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Evaluation of dietary approaches to improve growth performance of health-challenged pigs

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Evaluation of dietary approaches to improve growth performance of health-challenged pigs

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS

AA = amino acid(s)

ADFI = average daily feed intake

ADG = average daily gain

APP = acute phase protein

ASF = African swine fever

ATP = adenosine triphosphate

BW = body weight

Cal = calorie

CP = crude protein

Ct = cycle threshold

d = day

DC = dendritic cell

DE = digestible energy

DM = dry matter

DNA = deoxyribonucleic acid

DPI = days post inoculation

E. coli = *Escherichia coli*

EAA = essential amino acid

ELISA = enzyme-linked immunosorbent assay

g = gram

GE = gross energy

G:F = gain-to-feed ratio

HF = high fiber

HL = high lysine

IACUC = Institutional Animal Care and Use Committee

IFN = interferon

Ig = immunoglobulin

IL = interleukin

ISUVDL = Iowa State University Veterinary Diagnostic Laboratory

kg = kilogram

LE = low energy

LPS = lipopolysaccharide

LS = least squares

Lys:ME = grams of SID lysine-to-megacalories metabolizable energy ratio

ME = metabolizable energy

MHC = major histocompatibility complex

MHP = *Mycoplasma hyopneumoniae*

MJ = mega joule

MLV = modified live vaccine

N = nitrogen

NDF = neutral detergent fiber

NE = net energy

NEAA = non-essential amino acid

NK = natural killer cells

NRC = national research council

PCAI = post cervical artificial insemination

PAMP = pathogen associated molecular patterns

PBS = phosphate buffered solution

PCV = porcine circovirus

PD = protein deposition

PMN = polymorphonuclear neutrophils

PRDC = porcine respiratory disease complex

PRR = pattern recognition receptor

PRRS = porcine reproductive and respiratory syndrome

PRRSV = porcine reproductive and respiratory syndrome virus

RNA = ribonucleic acid

RT – PCR = real-time polymerase chain reaction

S:P = sample-to-positive ratio

SAS = statistical analysis system

SEM = standard error of mean

SID = standardized ileal digestibility

SIV = swine influenza virus

SLA = swine leukocyte antigen

SBM = soybean meal

Th = T-helper cells

TLR = toll-like receptor

TNF = tumor necrosis factor

Vac- = non-vaccinated

Vac+ = vaccinated

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (**PRRSV**) is one of the most economically significant pathogens in the U.S. today. The disease associated with this virus is Porcine reproductive and respiratory syndrome (**PRRS**), which antagonizes all stages of production by causing increased morbidity, mortality and reduced growth. Controlling the spread and eradication of this endemic pathogen has remained challenging due to the ease of transmission between animals, the various viral strains that exist, and varying vaccine efficacies. Thus, interest in nutritional strategies to help mitigate the negative growth performance phenotypes typically associated with a PRRSV challenge in growing pigs is rising. One nutritional strategy is to modulate the ratio of dietary amino acids (**AA**) to energy. Increasing standardized ileal digestible Lys per Mcal metabolizable energy (**SID Lys:ME**) above the requirement of healthy pigs has been reported to help mitigate reduced growth performance during a PRRSV challenge. Thus, the overall objective of this thesis was to evaluate the importance of increasing dietary SID Lys:ME above a growing pig requirement (i.e. targeting 120% of requirement) in pathogen challenged pigs to improve growth performance. Further, we also evaluated the formulation approaches used to achieve this increased ratio in PRRSV challenged pigs. To address this overarching thesis objective, a series of experiments were conducted and are outlined in two research chapters (Chapters 2 and 3).

In Chapter 2, the first experiment was conducted to evaluate the effects of increasing SID Lys:ME on growth performance in PRRSV vaccinated or nonvaccinated pigs facing a subsequent PRRSV challenge. Additionally, we evaluated the formulation approach used to achieve an increased SID Lys:ME (i.e. 120%), by either increasing SID Lys and other essential AA relative to energy, or by diluting energy relative to Lys. In Chapter 3, a second experiment

was conducted to further evaluate the formulation approach used to achieve a 120% Lys:ME ratio in PRRSV challenged grower pigs, by utilizing an industry applicable feedstuff to reduce dietary energy. Although an increase in Lys:ME has been shown to be beneficial in PRRSV challenged pigs, a third experiment (Chapter 2) was conducted to assess if an increased Lys:ME ratio would be beneficial to growth performance in *Mycoplasma hyopneumoniae* (**MHP**) challenged late-finishing pigs.

The results from this research validates previous work that increasing dietary SID Lys:ME to 120% of grower pig's requirement during a PRRSV challenge aided in mitigating negative growth performance associated with a PRRSV challenge (Chapter 2 and 3). Additionally, irrespective of PRRSV vaccination status, diluting ME by ~20% with sand (inert feed ingredient) to achieve a 120% Lys:ME, resulted in increased feed intake throughout the PRRSV challenge period, which translated to an increased ADG and end BW compared to the 100% Lys:ME control (Chapter 2). Thus, these data highlighted that pigs attempt to eat to their energy needs, even while undergoing a severe health-challenge. Utilizing dietary fiber to achieve the targeted 120% Lys:ME in the diets (Chapter 3), an increase in feed intake was observed in pigs fed the reduced ME diet, which was achieved via soybean hulls inclusion. However, the increase in feed intake did not translate into increased overall ADG or end BW compared to 100% Lys:ME control (Chapter 3).

Results of the third experiment, also outlined in Chapter 3, reported that increasing Lys:ME to 120% of requirement had no effect on growth performance in late finishing pigs challenged with MHP. However, in non-MHP challenged pigs, increasing Lys:ME also had no bearing on late-finishing growth performance. These data suggest that pigs undergoing a bacterial MHP challenge may not benefit similarly to increased Lys:ME ratios as previously

reported in virally challenged pigs or that increased Lys:ME is less important to support lean tissue growth in late-finishing.

In summary, this thesis concludes that both viral and bacterial health-challenges alter nutrient requirements of growing pigs as apparent by reduced pig performance during health-challenges. In PRRSV challenged pigs, this work herein validates that increasing SID Lys:ME to 120% of requirement augmented growth performance during the peak disease period of a PRRSV. Additionally, if pigs are fed a diet diluted in energy, these pigs will eat to their energy needs, even while undergoing a health-challenge. This increase in ADFI then translated to increased ADG and end BW in comparison to 100% Lys:ME fed pigs. However, increasing the Lys:ME ratio in bacterial challenged late finishing pigs resulted in no improvement in growth performance. Thus, indicating that feeding a diet containing Lys above requirement during a bacterial challenge in late finishing may not hold the same beneficial effects as previously seen in virally challenged grower pigs.

CHAPTER 1. LITERATURE REVEIW

Introduction

Optimal growth performance and lean tissue deposition of growing pigs can be largely dictated by the composition of dietary nutrients and energy in addition to various environmental and biological factors. Nutrients can be defined as chemical substances present in food that are necessary for maintenance of the body and for growth, lactation or gestation. Thus, nutrients include proteins, fats, carbohydrates, vitamins, minerals and water. However, energy is not considered a nutrient as it is not a chemical substance, but rather characteristic of the diet or nutrient. Nevertheless, energy is required for all biological processes in pigs. Nutrients contain chemical energy that is released upon chemical breakdown (metabolism) and this energy can then be used in the body to perform chemical, mechanical, electrical or osmotic work such as maintaining membrane potential in cells. Having the correct energy concentration in a pig's diet is of importance, as energy concentration in the diet affects many aspects of pig production, such as feed intake and feed efficiency (Patience, 2012). In an effort to optimize performance, there are effective levels of formulated dietary energy for the various stages of production as outlined by the National Research Council (**NRC**, 2012). Protein, or more specifically amino acids (**AA**), are also an important component of the diet. There is not a protein requirement per se for pigs, rather diets are formulated on an essential AA requirement basis instead. In healthy pigs, lysine (**Lys**) is the first limiting AA when feeding a corn-soybean based diet. Furthermore, the ratio of Lys to energy and other essential AA in the diet is also critical in optimizing growth (Smith et al., 1999). Interestingly, although much is known about the nutrient requirements of healthy pigs, AA and energy utilization and requirements for swine with an activated immune system are less well understood (NRC, 2012).

Advances in diagnostic and animal population management, biosecurity, and the production of replacement breeding stock free of common pathogens have greatly elevated the health of herds. However, diseases such as Porcine reproductive and respiratory syndrome (**PRRS**) still hold economic significance to the swine industry (Holtkamp et al., 2013; Nathues et al., 2017). The PRSS virus (**PRRSV**) is the infectious viral agent of PRRS that antagonizes all stages of production causing increased morbidity, mortality and reduced growth in swine (Lunney et al., 2010). Surprisingly, even with endemic diseases such as PRRS, feeding and managing these disease-challenged pig flows (populations), as well as knowing their nutritional requirements for health recovery and growth performance, have remained elusive.

This thesis literature review will examine and discuss nutrition by health concepts in growing pigs. More specifically, this review is divided into four main sections. The first section discusses energy and what factors are considered when determining dietary energy requirements of a growing pig. The second section reviews and discusses concepts of AA and crude protein requirements and utilization in growing pigs, as well as the importance of using AA to energy ratios in diet formulation. The third section discusses PRRSV infection and other common health-challenges that pigs face. As well as an overview of how the pig coordinates an immune response to pathogens and more specifically PRRSV. Lastly, the final section reviews and discusses pig performance outcomes under health-challenges and nutritional mitigation strategies that have been used to augment performance of PRRSV challenged pigs.

Defining Energy and Energy Utilization

All living organisms require energy for life processes such as the biosynthesis of proteins, bones and lipids and for the chemical processes associated with maintenance, for active ion

transport, membrane potential and for mechanical work such as movement of the body (Patience et al., 2015). The first law of thermodynamics states that energy cannot be created or destroyed; thus, in the body all energy must be acquired from ingested foodstuffs or from tissue stores. From an animal nutritionist standpoint, the definition of energy with the most relevance is that of the calorie (**cal**) or mega joule (**MJ**). A calorie can be defined as the basic unit of measuring energy; one calorie, or 0.00418 MJ, is the quantity of heat required to raise the temperature of water from 25.5˚C to 26.5˚C at one atmosphere of pressure from 1 g of material, i.e. feed (Russo and Silver, 2011). When analyzing the energy content of a diet, the caloric content of the ingredient or complete feed is used to predict the quantity of energy that will either be retained or utilized by the body. However, energy is a complex component within the diet as it is supplied in various forms (i.e. protein, fat, fiber, starch, etc.), and the metabolic pathways that transform each source into usable energy (adenosine triphosphate or **ATP**) also differ.

When nutritionists study energy, they need to consider that energy comes from four different dietary sources: 1) simple carbohydrates (primarily starch), 2) complex carbohydrates commonly referred to as fiber, 3) fat and lipids, and 4) protein. These sources of energy (Figure 1.1) are all carbon-containing compounds found in feed that release energy following digestion and metabolism or their energy can be stored in tissues such as skeletal muscle and adipose tissue. However, these dietary sources vary in rate of metabolic efficiencies in which the animal can utilize them along in addition to differing bioavailabilities (NRC, 2012). It is estimated that the complete combustion (oxidation) of one gram of a carbohydrate, lipid or protein yields 4, 9 and 5 calories, respectively (Jurgens, 2007). In terms of energy, metabolic efficiencies of nutrients vary depending on where the energy is being partitioned. Energy within the body can

be partitioned for maintenance, lactation or growth, with the latter being primarily retained as lean or adipose tissue.

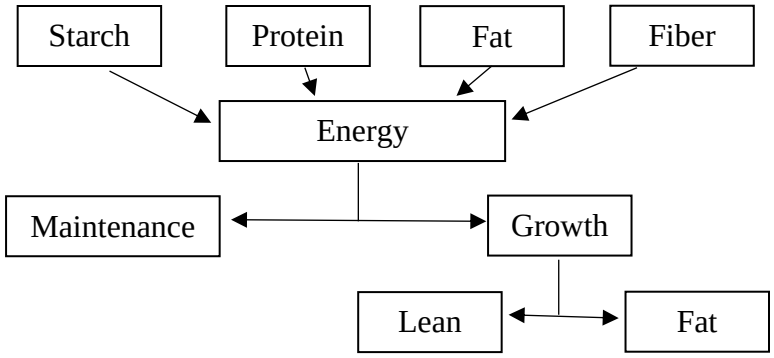

Figure 1.1. The four distinct sources of energy supplied by the diet. Adapted from Patience, 2012.

The energy system in which animal nutritionists formulate diets in is also an important aspect to consider when discussing energy. Energy systems fill two main roles. First, they provide a method to assign economic and nutritional value to feedstuffs. Secondly, these systems support the formulation of the diets to result in predictable performance when consumed by the pig (Patience, 2012). Once feed is consumed by the pig it has multiple fates; however, it cannot be destroyed. It can be absorbed into the body, stored in tissues, excreted in feces or urine or dissipated as heat (Kil et al., 2013). To account for these different fates, various energy systems (Figure 1.2) are used throughout the world as there is no single system that has captured the full agreement of all nutritionists.

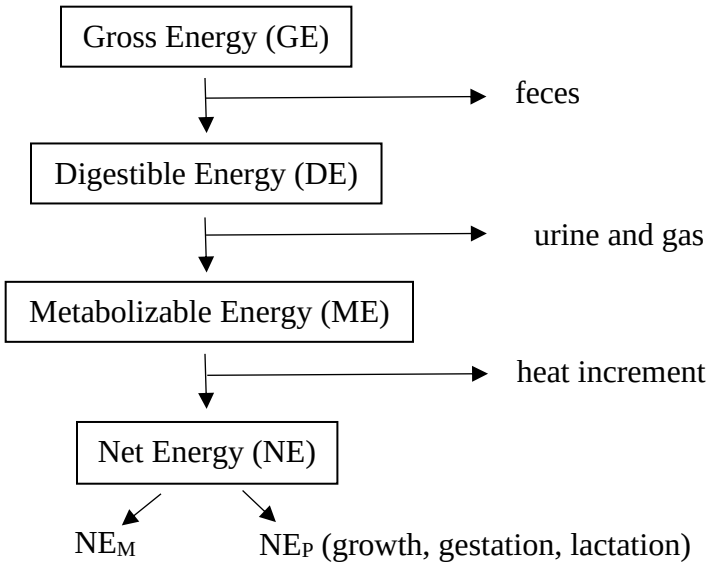

Figure 1.2. Partitioning of nutrient and dietary energy. Adapted from (NRC, 2012).

Gross energy (**GE**) is a measure of the total energy content in the feed ingredient. Gross energy can be determined through bomb calorimetry in which the total sample is completely combusted, with the only material left behind being inorganic. The value determined through bomb calorimetry can then be the basis for determining other energy values. However, the GE value provides no information about the amount of energy that is available to the pig after digestion and metabolism, thus more precise measurements are used by nutritionist. Digestible energy (**DE**) is simply GE corrected for the energy lost in feces. The energy excreted in feces contains indigested feed components, metabolic products and microbial material. Metabolizable energy (**ME**) also accounts for the energy lost in urine and gas, which is not taken into consideration when determining DE. A further refinement of ME is net energy (**NE**), that accounts for the energy lost through digestive processes and nutrient metabolism (i.e. heat production). The NE system accounts for the differences in metabolic utilization of ME between

nutrients; thus, the only system in which energy supplied by the diet and energy requirements of the animal are expressed on the same basis (Noblet and Henry, 1993). The DE or ME of feedstuffs is important in predicting NE values, but dependent also on the age of pig as the ability to digest certain nutrients especially fiber is age related (Kil et al., 2013). Additionally, if the chemical composition of the feed ingredient is known, energy values can be predicted using various equations (NRC, 2012). In North America the ME system is primarily used amongst swine nutritionists in diet formulation, while the NE system is slowly gaining popularity.

Energy Metabolism

In the body, energy metabolism can be broken down into two main categories consisting of catabolism and anabolism. Catabolism produces energy via oxidation of carbon containing molecules that result in ATP production, while anabolism synthesizes body components in endergonic reactions (Buron, 2009). Anabolism uses energy to build components of the cell such as protein, lipids and nucleic acids. If anabolism exceeds catabolism in the body, growth or body weight gain occurs. The growing pig has a requirement for energy that can be defined as energy required for maintenance, thermoregulation, mechanical work, protein deposition and lipid accretion (Noblet et al., 1999). Maintenance energy is that of the pig that is not associated with protein and lipid gain, therefore including body functions such as basal metabolism, normal protein turnover and nutrient digestion and absorption. Based on the NRC (2012), maintenance energy requirement (kcal per day [**d**]) can be calculated utilizing a pig's body weight in kg and by using the following equation:

$$ME_{maintenance}\,(kcal/d) = 197 \times BW^{0.60}$$

Thermoregulation, immune function, and stressors know to stimulate stress hormones (i.e. cortisol) that the pig may encounter also require energy which commonly falls into the maintenance energy category or is included as an adjustment (Knap, 2009). Once the energy requirement for maintenance has been met, the pig then diverts the "surplus" energy to lean and adipose tissue hypertrophy and overall body growth. In the pig, lean gain is more energetically efficient than lipid gain due to lean tissue containing a large portion of water (~60 to 70%); thus, protein gain requires about 10.03 kcal ME per gram of gain compared to 11.65 kcal ME per gram of lipid gain (Patience, 2012). However, it is important to remember that sufficient AA must be available for the pig to build lean tissue. Furthermore, pigs will reach a point at which they have reached their genetic capacity for lean tissue growth and beyond this point energy supplied will predominately be partitioned to lipid deposition (Velayudhan et al., 2015).

Providing insufficient energy and nutrients to the pig can limit growth, while feeding excess nutrients increases the cost of production as well as increasing nutrient excretion such as nitrogen. For this reason, phase feeding is often utilized in growing pigs in an effort to match dietary nutrient and energy supply with pig demand as closely as possible, improving overall production efficiency. Nutrient requirements change continuously with age (NRC, 2012). The effective ME content of the diet for pigs (Table 1.3) weighing 5 to 11 kg is 3,400 kcal per d, while for 11 to 25 kg pigs it is 3,350 kcal per d and 25 to 135 kg should be supplied 3,300 kcal per d according to NRC (2012) recommendations. Pigs of lower weight have an increased energy requirement due to various factors such as large relative heat loss to the environment and rapid growth and development. Older, larger animals with a decreased surface area to mass ratio have a slightly reduced energy requirement due to less heat being lost in the environment (van Milgen and Noblet, 2003). However, pigs have the ability to eat to their energy needs if energy is

supplied in sufficient amounts. Thus growing pigs are commonly given unrestricted access to feed, allowing for maximum growth performance (Baker et al., 1968; Noblet et al., 1994).

Another important aspect of energy concentration in feed ingredients is that the amount of energy in feed influences voluntary feed intake of pigs. It has been established that pigs will eat to their energy needs (Noblet et al., 1994). Thus, energy concentration of the diet will alter feed intake and efficiency. Baker et al. (1968) concluded that total feed intake and feed efficiency decreased as dilution level in the diet increased (i.e. reducing total dietary energy) up to a 20% inclusion of sand (i.e. a high-density inert diluent). A low-density diluent of purified cellulose was also utilized in this study as well. However, an increase in feed intake was not observed as energy was diluted via a high fiber ingredient, due to pigs having a limited ability to metabolize high fiber feed ingredients, in addition to gut fill.

Schinckel et al. (2012) also reported that pigs fed a low energy diet consisting of a 7.6% reduction in ME in comparison to the high energy diet resulted in a 6.9% increase in feed intake compared to the high energy fed pigs. Interestingly, the pigs fed the low energy diet had nearly identical ME and NE intakes as pigs fed the high energy diet, highlighting the pig's ability to eat to their energy needs. To avoid any confounding results, when formulating diets with differing energy ratios in a comparison study, Lys to energy ratios are kept constant across diets in addition to most other essential AA set to a minimum ratio to Lys (Table 1.1). This technique in formulation of swine diets emphasizes the importance of AA levels and/or ratios in the diet.

Table 1.1. Recommended NRC (2012) essential AA to lysine ratios for growing pigs

	Pig body weight range (kg)						
Item	**5 to 7**	**7 to 11**	**11 to 25**	**25 to 50**	**50 to 75**	**75 to 100**	**100 to 135**
SID Lys, % diet	1.50	1.35	1.23	0.98	0.85	0.73	0.61
SID AA:Lys							
Met+Cys:Lys	0.55	0.55	0.55	0.56	0.56	0.58	0.59
Thr:Lys	0.59	0.59	0.59	0.60	0.61	0.63	0.66
Trp:Lys	0.17	0.16	0.16	0.17	0.18	0.18	0.18
Ile:Lys	0.51	0.51	0.51	0.52	0.53	0.53	0.54
Val:Lys	0.63	0.64	0.63	0.65	0.65	0.66	0.67
Leu:Lys	1.00	1.00	1.00	1.01	1.00	1.01	1.02
His:Lys	0.34	0.34	0.34	0.35	0.34	0.34	0.34
Phe:Lys	0.59	0.59	0.59	0.60	0.60	0.60	0.61

Derived from the NRC (2012)

Crude Protein and Amino Acids in Growing Pigs

The main function of AA is to synthesize proteins, specifically the proteins incorporated into building muscle or lean tissue (Rezaei et al., 2013). Amino acids are commonly referred to as the building blocks of proteins because AA form short polymer chains, peptides and polypeptides that ultimately lead to the production of proteins. Under different physiological conditions dietary AA requirements in growing pigs vary depending on metabolic demand that must be met for maintenance, protein synthesis, and lean tissue accretion (Humphrey and Klasing, 2004). Dietary protein is typically assessed in the form of crude protein (**CP**). Protein is one of the most expensive nutrients in swine diets; thus, it is important to understand the physiological roles of AA in growth, development and health of pigs to provide adequate

nutrition. To balance conflicts that arise between nutrient requirements and least cost formulating, efficient use of dietary AA is crucial.

Over 300 AA occur in nature, yet there are 20 primary AA that are incorporated into proteins (Wu, 2009), which are simply nitrogenous organic compounds that are an essential part of living organisms. Each of the 20 AA contains a basic amino group (-NH2), a carboxyl (-COOH) functional group and a unique side chain or *R* group, specific to each AA. Of the 20 AA, all are chiral with the exception glycine due to its -H sidechain and are classified as D- or L-isomers. The L-isomer is the form in which most AA occur in plant and animal proteins and are generally utilized easier than D-isomers; however, this varies between species (Baker, 2006). When discussing AA, they are commonly classified into two groups, essential and non-essential AA and are commonly referred to by a one or three letter abbreviation (Table 1.2). For the continuation of this review these abbreviations will be used when discussing AA as well as essential AA (**EAA**) and nonessential AA (**NEAA**).

Table 1.2. Essential, conditionally essential, and nonessential amino acids

Essential	Conditionally Essential	Nonessential
Histidine, His, H	Arginine, Arg, R	Alanine, Ala, A
Isoleucine, Ile, I	Cystine, Cys, C	Asparagine, Asn, N
Leucine, Leu, L	Glutamine, Gln, Q	Aspartate, Asp, D
Lysine, Lys, K	Proline, Pro, P	Glutamate, Glu, E
Methionine, Met, M	Tyrosine, Tyr, Y	Glycine, Gly, G
Phenylalanine, Phe, F		Serine, Ser, S
Threonine, Thr, T		
Tryptophan, Trp, W		
Valine, Val, V		

Shown as AA full name, three-letter abbreviation, and one-letter abbreviation
Adapted from the (NRC, 2012)

An EAA is an indispensable AA whose carbon backbone cannot be synthesized by the animal and must be provided in the diet (Wu, 2010). Lysine, Met, Trp, Thr, Val, Ile, Leu, His and Phe are considered EAA in swine. Nutritional EAA such as Met, Phe and other branched chain AA can be synthesized by transamination of their analogous α-keto acids; however, these precursors are not usually available in sufficient quantities in pigs. Thus, formulated diets must contain sufficient levels of these EAA and nitrogen to synthesize required NEAA to meet the metabolic demand of maintenance, growth and/or reproduction. NEAA are assumed to be synthesized at sufficient rates to support normal physiological function when adequate amounts of non-specific protein and EAA are present. Some NEAA are considered conditionally essential under certain conditions such as stage of growth, reproductive function, disease state or dietary composition. Meaning that utilization rates of NEAA are above the rate at which the pig can synthesize these AA. These include Arg and Pro in freshly weaned pigs used to maximize protein synthesis (Ball et al., 1986) and Cys, Tyr, and Glu during disease or weaning stress used to support immune function (Rezaei et al., 2013).

As discussed, the primary function of dietary AA is protein synthesis; however, other important roles have also been revealed. For example, individual AA such as Gln and Arg, have been discovered to act as signaling molecules that regulate mRNA translation efficiency in animals (Anthony et al., 2000). Lysine, Gln and Asp are precursors for purine and pyrimidine bases used in the synthesis of deoxyribonucleic acid (**DNA**) and ribonucleic acid (**RNA**). Although lower in efficiency, AA can also be utilized as an energy source through oxidation, yielding ATP in incidents of energy deficiency, which commonly occurs when AA are in excess (Wu, 2009).

Additionally, various AA can also be used as a source of energy via the production of glucose (via. gluconeogenesis) or ATP directly. This dual role of AA during metabolism, and the fact that protein synthesis is an energy demanding process, is the basis of protein-energy interaction during growth (Moughan, 2018). The swine industry has evolved from DE or ME to the use of NE in diet formulation. Now diets are formulated on a Lys to energy ratio and other AA are balanced accordingly as a ratio of the AA of interest to Lys (Wang and Fuller, 1989). The current NRC (2012) recommendations for g standardized ileal digestible (**SID**) Lys:ME are shown in Table 1.3. Considering the unrestricted feeding practices in today's swine industry as well as the pig's ability to eat to their energy needs, formulating AA on a ratio to energy is a way for nutritionists to ensure formulated diets contain sufficient levels of AA for growth. These changes in technology and diet formulation strategies have benefited pork producers through improved growth rate, feed efficiency and carcass leanness while reducing feed costs per pound of gain. However, limited research has been conducted to determine if the optimal g SID Lys:ME differs in health-challenged pigs.

Table 1.3. Recommended NRC (2012) g SID Lys:ME for growing pigs

	Body Weight Range (kg)						
Item	**5 to 7**	**7 to 11**	**11 to 25**	**25 to 50**	**50 to 75**	**75 to 100**	**100 to 135**
SID Lys, % diet	1.50	1.35	1.23	0.98	0.85	0.73	0.61
ME kcal/kg diet	3,400	3,400	3,350	3,300	3,300	3,300	3,300
g SID Lys:ME	4.41	3.97	3.68	2.97	2.58	2.21	1.85

Derived from the NRC (2012)

Health-Challenges Facing Pigs

At some point in their lifetime, pigs will be exposed to pathogens that pose a moderate to severe health-challenge. Modern pig production in a confinement setting with a common airspace has provided the ideal environment for horizontal transmission within dense pig populations via fecal or aerosol contamination and often direct contact with an infected pig (Murtaugh et al., 2010). On a global scale, there are various swine pathogens that affect different areas of the world. A review of over 57,000 publications from 1966 to 2016 was conducted (VanderWaal and Deen, 2018), constructing the most important swine pathogens by regions based on this literature (Figure 1.3). In the U.S., common pathogens that swine may be exposed to are often bacterial, viral or a coinfection of the two (Table 1.4). Common bacterial infections to emphasize include *Streptococcus suis*, *Glaesserella parasuis*, *Mycoplasma hyopneumonie, Salmonella* and *Escherichia* coli, as well as a few common viruses including PRRSV, Porcine Circovirus (**PCV**) and Swine Influenza Virus

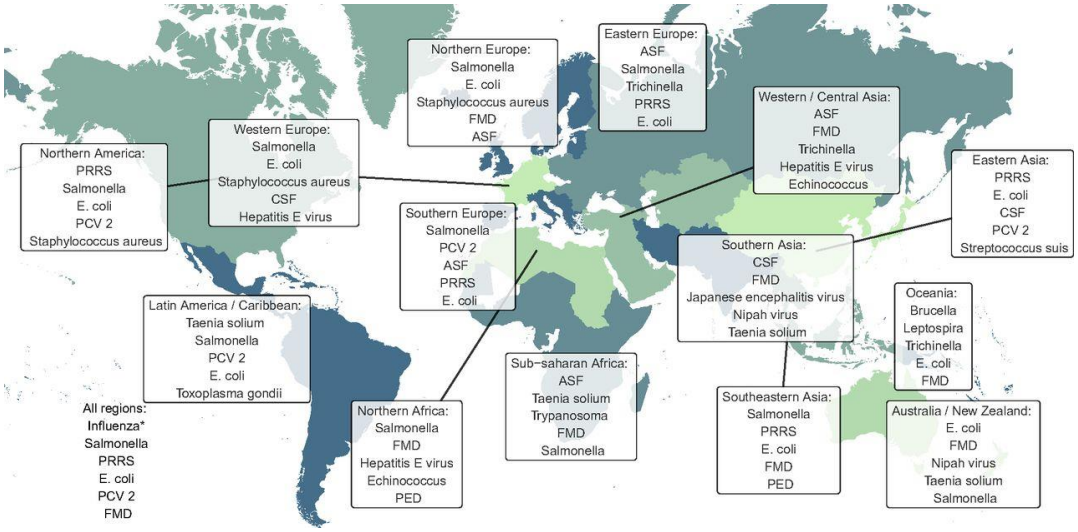

Figure 1.3. Most important pathogens of swine by region from 2006 to 2016, ranked in descending order by publication number. Asterisk indicates pathogens appearing on each regions list and thus excluded from regional lists. Source: (VanderWaal and Deen, 2018). Abbreviations in figure: ASF (African swine fever), CSF (Classical swine fever), E. coli (*Escherichia coli*), FMD (Foot and mouth disease), PCV2 (Porcine circovirus 2), PED (Porcine epidemic diarrhea) and PPRS (Porcine reproductive and respiratory syndrome)

(**SIV**). Nevertheless, with the exception of African swine fever (**ASF**), PRRSV is arguably one of the most economically significant health-challenges to the swine industry and has been associated with large economic losses to producers (Holtkamp et al., 2013; Nathues et al., 2017). In the U.S. alone it is estimated that PRRSV costs swine producers upwards of $644 million annually (Holtkamp et al., 2013). Thus, for the remainder of this review, we will only be discussing PRRSV and potential nutritional strategies that could be utilized to help mitigate this virus.

Table 1.4. Common pathogens to the U.S. swine finishing industry

Biological name	Common name	Type	Affected organ(s)
Streptococcus suis	Strep suis	Bacteria	Respiratory/ septicemia
Glaesserella parasuis	Glässer's disease	Bacteria	Respiratory/ septicemia
Actinobacillus suis	Asuis	Bacteria	Respiratory/ septicemia
Mycoplasma hyopneumoniae	Myco/ Mhyo	Bacteria	Respiratory/ airways
Escherichia coli	E. coli	Bacteria	Intestinal epithelium
Salmonella spp.		Bacteria	Intestine and colon
Lawsonia intracellularis	Ileitis	Bacteria	Intestine and colon
Brachyspira hyodysenteriae	Swine dysentery	Bacteria	Large intestine epithelium
Swine Influenza Virus	SIV/ flu	Virus	Bronchial epithelium
Porcine circovirus type 2	PCV2	Virus	Epithelia/endothelial cells multisystemic
Porcine epidemic diarrhea	PEDV	Virus	Intestinal enterocytes
Rotaviruses A, B or C	Rotavirus	Virus	Intestinal epithelium
Porcine respiratory coronavirus	PRCV	Virus	Lung
Porcine deltacoronavirus	PDCoV	Virus	Intestine
Porcine reproductive and respiratory syndrome virus	PRRSV	Virus	Respiratory tract / multisystemic

Adapted from The Merk Veterinary Manual (Aiello et al., 2016) and Diseases of Swine (Chase and Lunney, 2019)

Porcine Reproductive and Respiratory Syndrome

The first outbreaks of PRRSV in the U.S. was recorded in the late 1980s and in Europe in 1990 (Gilbert et al., 1997; Murtaugh et al., 2010; Holtkamp et al., 2013). Induced by the PRRSV,

PRRS can be characterized by reproductive failure in sows, including mummified, stillborn and aborted fetuses, as well as respiratory distress in any age of pigs (Goyal, 1993). Clinical signs of the respiratory syndrome associated with the PRRSV include lethargy, anorexia, dyspnea and rough hair coat. A PRRSV infection in pigs may increase the occurrence of secondary infections, mortality, morbidity, variation in herd body weights as well as antimicrobial administration, in an effort to mitigate secondary infections (Pileri and Mateu, 2016).

The causative agent of PRRS disease is PRRSV, a small, enveloped, positive strand RNA virus (Conzelmann et al., 1993). Soon after the discovery of PRRSV, it was determined that the European (type 1) and North American (type 2) strain are biologically different, only sharing 55-70% of nucleotide identity (Gilbert et al., 1997). Nucleic acid sequencing revealed that both were only distantly related, while also revealing genetic similarities in both virus strains to arteriviruses, equine arteritis virus, lactate dehydrogenase-elevating virus and simian hemorrhagic fever (Murtaugh et al., 2010). Type 1 and 2 PRRSV strains have shown to cause similar clinical signs; however, type 1 is often more associated with reproductive failure and abortions, while type 2 causes respiratory stress in growing pigs as well as reproductive failure and abortions in the breeding herd. Transmission of this virus is primarily through pig-to-pig contact; however, exposure to this virus can occur through various forms of indirect transmission including vectors, fomites, and aerosol transmission. Transmission of the virus is variable, as highly virulent isolates that are shedding high amounts of virus transmit at a higher frequency than less pathogenic viruses (Aiello et al., 2016). Now, to further understand how to best feed a pig undergoing a health-challenge such as PRRS, we must understand the immune response that occurs when the pig is exposed to the virus.

Immune System and Function of Growing Pigs

The immune system has evolved to protect the host from foreign material and pathogenic microbes which are also constantly evolving. An important attribute of the immune response to an invading pathogen is the ability to distinguish self from non-self, signaling to the immune system to avoid damaging self-tissues (Chaplin, 2010). The host will use two primary lines of defense in response to invading microbes.

The first line of defense is the innate immune system (non-specific defense), while the second line of defense is the adaptive or acquired immune system (specific defense). The innate immune system responds almost immediately to the introduction of foreign material or a pathogen, and the animal's acquired immune system is activated following the innate immune response (Figure 1.4). As anticipated, the innate and adaptive immune systems work together but are often described separately in their function. However, it is important to note that the collaboration between these two systems is essential for an effective immune response.

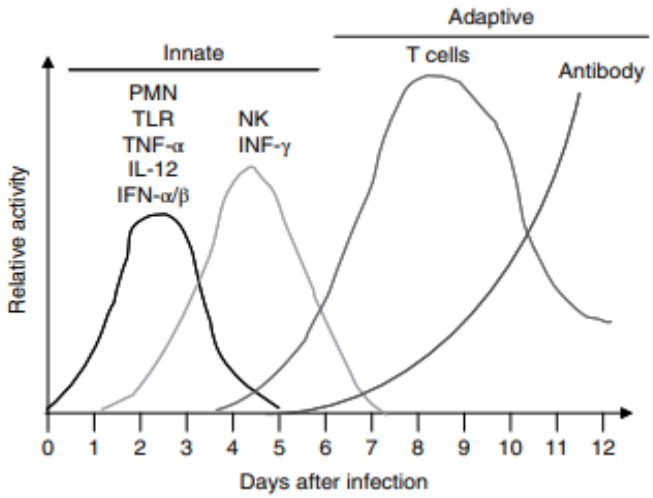

Figure 1.4. Timing of host response to infection: mobilization of innate and adaptive response. Source: Diseases of Swine 11th ed. (Chase and Lunney, 2019) Abbreviations in figure: polymorphonuclear neutrophils (PMN), toll-like receptors (TLR), tumor necrosis factor (TNF), interleukin (IL), interferon (IFN) and natural killer cell (NK).

The Innate Immune Response

The first line of defense against pathogens that involves the innate immune system is the physical and chemical barriers of the pig: epithelial cells, tight junctions, stomach acid, low pH environments, enzyme rich environments and mucosal barriers (attached and secreted). These defense barriers are important as the animal's skin, respiratory tract and gastrointestinal tract are the most common sites by which pathogens gain entry into the body, consequently activating the innate immune system response. In the case of PRRSV infection, the virus typically infiltrates the respiratory tract and replicates in porcine alveolar macrophages (Duan et al., 1997; Zhang and Yoo, 2015). Mucus present in the respiratory tract acts to trap and expel various pathogens via the mucosal ciliary escalator located in the respiratory tract. However, PRRSV can infiltrate this barrier, along with mucosal membranes present in the nasal cavity, oropharynx and lungs.

Once PRRSV is in circulation or in infected cells and tissues, the classic innate immune response to this viral pathogen is the recognition of pathogen-associated molecular patterns (**PAMPs**) on the pathogen by host pattern recognition receptors (**PRR**). Pattern recognition receptors induce different signaling pathways depending on what pathogen is detected, then triggering the appropriate immune response (Chase and Lunney, 2019). An example of a PRR is a Toll-like receptor (**TLR**), which are mainly located on the plasma membrane of the cells. The binding of viral or microbial components will lead to TLRs initiating an inflammatory response signaling to other components of the immune system. In pigs and in the case of PRRSV, TLR 7 and 8 are involved with the recognition of single stranded RNA viruses, as is found in PRRSV (Takeuchi and Akira, 2010).

The innate immune response encompasses the involvement of phagocytic cells and the production of various cytokines, chemokines and proteins that provide antimicrobial protection

as well as recruit T-cells through inflammation and activating the adaptive immune response. Inflammation is a protective response of the host, to ensure removal of the pathogen by initiating immune system stimuli as well as helping in the repair of damaged tissues. The inflammation response is orchestrated by pro-inflammatory cytokines such as tumor necrosis factor (**TNF**) and interleukin (**IL**)-1 and 6 being released from macrophages, and natural killer cells (**NK**) (Takeuchi and Akira, 2010). Macrophages are considered antigen presenting cells and play a vital role in the effector functions of immune response and maintenance of tissue homeostasis. These macrophages are able to kill intracellular pathogen by engulfing them and then producing pro-inflammatory cytokines (Crisci et al., 2019). An increase in the release of pro-inflammatory cytokines is common in health-challenged pigs, acting to reduce appetite and facilitate muscle protein degradation (Johnson, 1997; Escobar et al., 2004).

The NK cells, also of the innate immune system, have the ability to spontaneously attack the pathogen by lysing the infected target cell to help with the removal of virus infected cells from the body. However, it has been shown that PRRSV has had the ability to induce significant suppression of NK cell cytotoxic activity, with the magnitude of this effect being strain dependent (Lunney et al., 2016). Further, it has been shown that younger nursery pigs possessing a less developed innate immune systems have reduced NK cell cytotoxic activity as their NK cells lack adequate intracellular granules (Van Reeth et al., 1999). The regulation of NK cell function during a viral infection such as PRRSV is coordinated by multiple cytokines such as interferon (**IFN**)-α and β, IL-12 and 15. Unlike most other pathogens, PRRSV prompts only a mild to moderate IFN-α response in both the respiratory tract and the lungs of infect pigs. This reduction in IFN-α response effects the adaptive immune response as the pathway controlling

adaptive immunity, and the antiviral response is predominantly controlled by IFN-α (Loving et al., 2015).

The Adaptive Immune Response

While the innate immune system is functioning to eliminate the invading pathogen, there has also been signaling for the activation of the adaptive immune response by means of dendritic cells (**DC**), bridging the innate and adaptive immune response. In the event of a PRRSV challenge, it has become increasingly evident that the bridge between the innate and adaptive immune system occurs through the interaction of DC and type I IFN (Loving et al., 2015). The DC are important antigen-presenting cells (via phagocytosis of phagosomes) that prime naïve T cells, driving the adaptive immune response. The antigenic peptides that are being presented on the cell surface via major histocompatibility complex (**MHC**) class I molecules and MHC class II for viruses and bacteria, respectively (Crisci et al., 2019). These MHC complexes are also referred to in the literature as swine leukocyte antigen (**SLA**). T cells do not possess the ability to respond to free soluble antigens, whole bacteria or whole viruses, therefore MHC or SLA class I and II molecules play a large role in antigen presentation. Overall, the antigen presentation results in stimulation of the B cells and T cells initiating the adaptive immune response.

Clearance of viruses is mainly due to cytotoxic T cells such as CD8+ that will recognize antigen presenting cells and kill the intracellular pathogens (Chase and Lunney, 2019). In addition, the CD4+ T cell can help promote the action of CD8+ T-cells with the DC cells in secondary lymphoid tissue as well as recruiting antigen-specific effectors to the site of viral replication (Sant and McMichael, 2012). Both CD4+ and CD8+ T cells differentiate once

exposed to a pathogen from naïve to effector populations (Chaplin, 2010). Also, T helper (**Th**) cells are critical in initiating optimal B cell response, resulting in switching of antibody production from immunoglobulin (**Ig**) M to IgG, IgA or IgE. B cells are able to contact the pathogen through Ig's bound to their surface acting as B cell receptors. The T cells are important for humoral memory for the first naïve response but become less important in recurring responses. In the secondary responses, neutralizing antibodies play a larger role in protecting the host from infection (Rahe and Murtaugh, 2017).

A common neutralizing antibody found in the serum of pigs is IgG, whereas IgA is the major mucosal Ig produced in swine which aids in mucosal, gastrointestinal and respiratory challenges (Chase and Lunney, 2019). Although not fully understood, PRRSV has the ability to spread viral RNA to neighboring cells via intercellular nanotubules thereby avoiding neutralizing antibodies (Guo et al., 2016). However, PRRSV does not appear to attenuate B cell differentiation (humoral immunity) (Rahe and Murtaugh, 2017). Thus, adaptive B cell response is not delayed or suppressed due to PRRSV. The function of B cells is to produce antibodies, which are vitally important to immune response and function. Development of protective humoral immunity after exposure to a pathogen or vaccination is depend on secreted antibodies and memory B cells.

Despite the fact that PRRSV has been extensively researched for the last 30 years, it remains an economically significant pathogen in the swine industry. Also, the contribution of PRRSV to compromised modulation of the immune response favors secondary microbial infections and Porcine respiratory disease complex (**PRDC**), often leading to severe morbidity due to reduced host resilience. Examples of enhanced disease expression by common subclinical bacterial and viral infection following a PRRSV infection are, PCV2, *Salmonella enterica*,

Glaesserella parasuis, various *Mycoplasma* species and *E.coli* (Rahe and Murtaugh, 2017). Thus, a possible nutritional feeding strategy that could help mitigate reduced performance and overall disease incidence may be of interest to the producer.

Performance of Health-Challenged Pigs

To most efficiently feed healthy or health-challenged growing pigs, providing a well-balanced diet that is both palatable and bioavailable to the pig is vital. Thus, providing a diet that meets the nutritional needs of the growing animal is of great importance to the producer. Providing a diet that fails to offer all essential nutrients in adequate amounts to the pig may ultimately immune-compromise the pig and leave it more susceptible to infectious diseases (Patience, 2012). As discussed previously, infectious pathogens can trigger a vigorous host immune response that can lead to an array of metabolic changes. These metabolic changes may require different nutrient requirements to support optimal performance than of those of a healthy pig.

Disease research with live bacterial or viral pathogens in growing pigs can be difficult. However, inflammation is often a major component of bacterial and viral pathogen-induced disease. As such, endotoxin or lipopolysaccharide (**LPS**) mediated inflammation challenge models have been developed, derived from semi-purified or purified gram-negative bacterial cell walls. The objective of the LPS challenge model is to induce an acute systemic inflammation response via increased plasma levels of various pro-inflammatory cytokines, mimicking an immune response (Webel et al., 1997). These LPS challenge models have shown reductions in average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed efficiency (gain-to-feed,

G:F) by as much as 30%, 22% and 21%, respectively with the most dramatic reductions occurring in younger pigs (Dritz et al., 1996; Williams et al., 1997). While the LPS challenge model is a useful to induce an experimental immune challenge, to further understand the complete immune response live challenge models will be the focus for the remainder of this review.

When a pathogen has triggered a host inflammatory response, metabolically active cytokines are released, successively causing reduced appetite and feed intake, inhibited nutrient absorption, increased metabolic rate and altered nutrient utilization (Johnson, 2002). Pro-inflammatory cytokines that are released cause an array of changes in host metabolism including triggering the acute phase protein (**APP**) response. The APP response is an innate (non-specific) immune response that can be induced due to events such as infection, tissue damage, or immunological disorders (Sorensen et al., 2006). Acute phase proteins are indicators of inflammation and stress, and are induced by pro-inflammatory cytokines such as IL-1, IL-5, and tumor necrosis factor α (**TNF-α**) (Sorensen et al., 2006). The stimulation of the immune system signaling for synthesis of APP could influence a shift in AA and protein metabolism in health-challenged pigs away from lean tissue accretion, as this process requires both free AA and energy. Thus, the metabolic and nutritional cost of immune stimulation is evident, illustrating the importance of defining nutrient requirements for pigs undergoing a health-challenge, specifically PRRS.

The severity of PRRS is dependent on two key factors. First, the age of the pig as younger growing pigs are usually more severely impacted by a PRRSV infection (Van Reeth et al., 1999). Secondly, the viral strain is a key factor as some strains are more virulent than others (Kappes and Faaberg, 2015). It is well established that in the face of a PRRSV challenge,

reductions in ADG and ADFI are often reported while impacts on feed efficiency (G:F) have been variable (Table 1.5). Variable observations of feed efficiency in PRRSV challenged pigs may be a consequence of reductions in ADFI often contributing to a reductions in lean tissue accretion (Schweer et al., 2017). Furthermore, continuous activation of the immune system also contributes to suppressed muscle growth (Johnson, 1997). Additionally, reductions in appetite during a disease challenge results in reduced nutrient intake and availability for tissue hypertrophy (i.e. skeletal muscle growth). Thus, the overall loss in growth during a PRRSV infection is the combined impact of indirect reduced feed intake and the direct effects of supporting the proper immune response, although the individual contribution of each are not well defined (Klasing et al., 1987).

During anorexia (i.e. disease associated anorexia), any change in AA needs can be met by the mobilization of endogenous AA stores found in protein, ultimately from skeletal muscle. (Reeds and Jahoor, 2001). Furthermore, reduced luminal nutrient and energy uptake during disease may trigger increased tissue mobilization, even in the absence of catabolic stimulus (Helm et al., 2019). In the event of a viral infection such as PRRSV, growth rates reductions have been reported to range from 19 to 135% lower, compared to that of healthy controls during peak viremia (Table 1.5). However in a PRRSV pair-fed study, additional losses were observed in PRRSV pigs than that of feed restricted, indicating the additional cost of immune stimulation (Helm et al., 2019). A commonly observed disease phenotype of PRRS is a febrile response, typically seen early on during the infection (Greiner et al., 2000, 2001; Che et al., 2011). Therefore, strategies to increases caloric (energy) intake and nutrient intake may be a nutritionally beneficial, in an effort to mitigate growth performance loss commonly seen.

Considering a healthy pig's ability to eat to its energy needs, it may be of interest to determine if this ability remains in a health-challenged state.

Table 1.5. Effect of PRRSV on growth performance parameters compared to a PRRSV naïve pig

Challenge	BW or Age	Strain/Isolate	Duration	Changes from Naïve Control	Reference
PRRSV	5 wks	High virulence isolate P-129	14 d	ADG ↓ 59% ADFI ↓ 43% G:F↓ 29%	(Che et al., 2011)
PRRSV	6 wks	High-virulence isolate P-129	14 d	ADG ↓ 43% ADFI ↓ 43% G:F ↔	(Escobar et al., 2004)
PRRSV	11 kg	ORF5 RFLP 1-3–4	17 d	ADG ↓ 104% ADFI ↓ 49% G:F ↓ 125%	(Helm et al., 2020)
PRRSV	9 kg	P-129 isolate	14 d	ADG ↓ 42% ADFI ↓ 30% G:F ↓ 18%	(Rochell et al., 2015)
PRRSV	13 kg	ORF5 RFLP 1-3–4	17 d	ADG ↓ 135% ADFI ↓ 50% G:F ↓ 135%	(Helm et al., 2019)
PRRSV	16 kg	ORF5 RFLP 1-18-4	21 d	ADG ↓ 30% ADFI ↓ 25% G:F ↔	(Schweer et al., 2016)
PRRSV + PEDV	16 kg	ORF5 RFLP 1-18-4	21 d	ADG ↓ 46% ADFI ↓ 42% G:F ↓ 23%	(Schweer et al., 2016)
PRRSV	34 kg	ORF5-RFLP 1–18–4	42 d	ADG ↓ 19% ADFI ↓ 10% G:F ↓ 12%	(Schweer et al., 2017)

Increased (↑), decrease (↓) no change (↔)

Nutritional Disease Mitigation Strategies

In the context of feeding pigs with PRRS, increasing the dietary inclusion of soybean meal has been proposed to promote earlier viral clearance and recovery as well as enhancing pig

performance and wellbeing (Boyd, 2014; Rochell et al., 2015). With soybean meal serving as the primary protein/AA source in corn-soybean meal-based swine diets, altering the inclusion level of this feed ingredient to better suit the metabolic needs of pigs undergoing a health-challenge is of interest. Rochell et al. (2015) reported that increasing soybean meal from 17.5% to 29% in the diet of nursery pigs reduced viremia load and improved growth during a 14 d PRRSV infection. The authors attributed the improved performance to various component of the increased soybean meal diet including increased concentration of CP and AA or the increase in bioactive antioxidant-compounds (i.e. isoflavones). However, the latter has yielded mixed result in PRRSV challenged pigs (Greiner et al., 2000; Smith et al., 2019). In a recent PRRSV challenge study conducted by Schweer et al. (2018b), evaluating the benefits of feeding an increased soybean-meal diet has shown that the potential benefits are likely not directly related to the digestibility of the nutrients. However, minimal differences in SID values of most AA were observed when comparing basal endogenous losses of AA in PRRSV challenged pigs to a healthy control group (Schweer et al., 2018b). Basal endogenous loss of AA represents the quantity of AA inevitably lost by the pig in relation to the flow of feed through the digestive tract or the metabolic state of the animal (Stein et al., 2007). These contradictive findings point towards the benefit of evaluating the effects of changing specific nutrient levels of the diet, to better understand possible benefits of altering nutrient levels to better match the requirements of a health-challenged pig.

When looking at disease nutrition research focusing on a specific nutrient, AA are commonly an area of interest. Amino acid requirements of health-challenged pigs have primarily focused on Lys, Met, Thr, and Trp as those AA are commonly the first four limiting AA in a healthy pig's typical corn-soybean based diet. However, studies have shown that the order of

limiting AA may be altered in health-challenged pigs (Reeds et al., 1994; Reeds and Jahoor, 2001). A limiting AA is an EAA present in the diet in a lower amount than what is needed to support protein synthesis. If deficient, synthesis of APP will not proceed beyond the rate at which that AA in available. Reeds et al. (1994) reported that the first limiting AA are Phe, Trp, and Ser, respectively, while Met, Thr, and Lys are incorporated into APP production in health-challenged pigs. Also, it has been show that Cys released from skeletal muscle is very similar to the theoretical requirements for the synthesis of APP (Reeds et al., 1994). In agreeance, the optimal Met:Met+Cys was increased in growing pigs challenged using a LPS modeled immune stimulation, showing the growing importance of Met and Cys in immune challenged pigs (Litvak et al., 2013). These findings indicate the importance of various AA and their limiting ability to regulate the immune response and growth unless adequately supplied in the diet during an immune challenge.

Keeping in mind that swine diets are often formulated on Lys to energy ratio, this ratio is also of interest in health-challenged pigs. To address Lys:ME needs of PRRSV challenged pigs, Schweer et al. (2018a) utilized breakpoint analysis to evaluate the effects of six graded levels of g SID Lys per Mcal ME. To create the graded levels of Lys:ME, soybean meal and synthetic Lys were increased, nevertheless maintaining EAA to Lys ratios. These authors concluded, that in the face of an experimental PRRSV challenge, increasing Lys:ME 110% to 120% above NRC requirement (100%) resulted in improved growth performance and feed efficiency. This study also reported that growth was optimized at similar total daily Lys intake in both control and PRRSV infected pigs. These results however differ from findings reporting that the Lys requirement (g/day basis) is reduced in immune stimulated pigs compared to non-immune stimulated pigs (Williams et al., 1997; Zimmerman et al., 1997). This is thought to be attributed

to increased lean tissue deposition in pigs with a low immune system activity compared to those with high immune stimulation. However, these findings show the importance of determining nutrient requirements of disease challenged pigs as they may alter due to increased immune function, protein catabolism or reduced feed intake.

Conclusions

In the world of integrated swine production and increasing regulations on antibiotic usage, health-challenges are inevitable in modern swine production. Knowing there is a metabolic cost of immune stimulation, changes in nutrient requirements of health-challenged pigs are needed, warranting further research.

An area of opportunity to better understand nutrient requirement of health-challenged pigs in that of Lys to energy ratios. To our knowledge only one study has evaluated the effects of graded levels of SID Lys:ME to determine the optimal ratio in PRRSV challenged pigs. Therefore, to better understand AA and energy utilization, specifically in PRRSV challenged pigs, the focus of this will be evaluating the effects of increasing the dietary SID Lys:ME above that of recommend requirements for grower pigs (Table 1.3) subjected to health-challenges. Additionally, limited research has been conducted evaluating energy levels in disease challenged pigs. Ultimately, finding practical dietary formulation of AA and energy that abate the poor growth performance health-challenged pigs is desired.

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CHAPTER 2. INCREASING THE RATIO OF SID LYSINE TO METABOLIZABLE ENERGY IMPROVES PIG PERFORMANCE DURING A VIRAL CHALLENGE

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Abstract

Porcine reproductive and respiratory syndrome virus (**PRRSV**) compromises pig performance. However, increasing standardized ileal digestible Lys per Mcal metabolizable energy (**SID Lys:ME**) above requirement has been shown to mitigate reduced performance seen during a PRRSV challenge. The objective of this study was to evaluate the effects of increasing the dietary SID Lys:ME from 100% National Research Council (**NRC**) requirement to 120% of the requirement in vaccinated **(vac**+; modified live vaccine [MLV] Ingelvac PRRS®) and non-vaccinated (**vac-**; no PRRS vaccine) grower pigs subjected to a PRRSV challenge. In addition, the dietary formulation approach to achieve the 120% ratio by increasing Lys relative to energy

(**HL**) or diluting energy in relation to Lys (**LE**) was evaluated. This allowed us to test the hypothesis that pigs undergoing a health-challenge would have the ability to eat to their energy needs. Within vaccine status, 195 mixed sex pigs, vac+ (35.2 ± 0.60 kg BW) and vac- (35.2 ± 0.65 kg BW) were randomly allotted to one of three dietary treatments (2.67, 3.23, or 3.22 g SID Lys:ME) for a 42 d PRRSV challenge study representing 100, 120 and 120% of NRC requirement respectively. Pigs were randomly allotted across two barns, each containing 24 pens with 7-10 pigs per pen (8 pens/diet/vaccine status). On dpi 0, both barns were inoculated with PRRSV and started on experimental diets. Within vaccine status, weekly and overall challenge period pig performance were assessed. In both vac+ ($P < 0.05$) and vac- ($P < 0.05$) pigs, the HL and LE diets increased end BW and overall average daily gain (**ADG**) compared to pigs fed the control diet ($P < 0.05$). Overall average daily feed intake (**ADFI**) during the challenge period was greater ($P < 0.05$) for pigs fed the LE diet compared to pigs fed control and HL treatments, regardless of vaccine status (20% and 17% higher ADFI than the control in vac+ and vac- pigs, respectively). The HL vac+ pigs had the greatest feed efficiency (gain-to-feed, **G:F**) compared to the control and LE pigs (0.438 versus 0.394 and 0.391 kg/kg respectively; $P <0.01$). Feed efficiency was not impacted ($P > 0.10$) by treatment in the vac- pigs. In summary, PRRSV challenged grower pigs consumed feed to meet their energy needs as indicated by the increase in ADFI when energy was diluted in the (LE) diet, compared to control pigs. In both PRRS vac+ and vac- pigs subsequently challenged with PRRSV, regardless of formulation approach, fed 120% SID Lys:ME diets resulted in enhanced overall growth performance.

Keywords: lysine, metabolizable energy, porcine reproductive and respiratory syndrome virus, pig

Introduction

Porcine reproductive and respiratory syndrome (**PRRS**) is a disease caused by the PRRS virus (**PRRSV**) pathogen. This disease is arguably the most economically significant health-challenge to the swine industry (Holtkamp et al., 2013; Nathues et al., 2017) as it antagonizes all stages of production causing increased morbidity, mortality and decreased growth (Lunney et al., 2010). With moderate success, the swine industry has employed vaccine strategies to reduce the occurrence of PRRS in swine herds (Meng, 2000; Zuckermann et al., 2007; Renukaradhya et al., 2015). Commercially available vaccines, either modified live vaccines (**MLV**) or autogenous vaccines developed from indigenous field isolates, have been widely researched resulting in varying efficacy (Osorio et al., 1998 ; Mavromatis et al., 1999; Jeong et al., 2018). In today's swine industry, it is common practice for herds to be vaccinated against PRRSV in an effort to mitigate the negative growth performance anticipated by a PRRSV challenge. However, due to variable efficacy of PRRSV vaccines, nutritional strategies may also be an effective way to improve performance during a PRRSV challenge.

Nutritional requirements for healthy pigs are well established by the National Research Council (**NRC**, 2012); however, nutrient requirements for pigs undergoing a health-challenge are widely unknown, and this includes amino acids (**AA**). In a healthy pig, Lys is the first limiting AA when feeding corn-soybean meal-based diets. However, AA utilization for swine with an activated immune system is not as well understood (NRC, 2012). In practical diet formulation, AA requirements are expressed in relation to energy as a ratio (i.e. SID Lys:ME). This ensures that a constant AA intake is achieved by the pig independent of the dietary energy level fed and related adjustment to feed intake, which is key to support optimal feed intake and growth. However, stimulation of the immune system due to a pathogen challenge can result in reduced

voluntary feed intake and as a result lower energy and AA intake (Johnson, 2002; Doeschl-Wilson et al., 2009) that causes growth rate reductions (Greiner et al., 2001; Rochell et al., 2015; Schweer et al., 2018a). Furthermore, it has been suggested that under unrestricted feed conditions, healthy pigs will attempt to consume the amount of feed required to satisfy their requirement for energy and nutrients (Schiavon et al., 2018). However, it is unclear if pigs can adjust their feed intake to meet their energy needs under stress or disease.

Nutritional strategies have previously been studied to promote earlier viral clearance and recovery that also enhance pig performance and well-being. One strategy has been to increase dietary soybean meal (Boyd, 2014; Rochell et al., 2015). Soybean meal is the primary dietary protein and amino acid source in traditional corn-soybean meal-based swine diets. It has been reported that increasing soybean meal from 17.5% to 29% reduced viremia load and improved growth in PRRSV infected nursery pigs in an experimental setting (Rochell et al., 2015). However, it is unclear if the improved performance is due to increased concentration of crude protein (**CP**) and AA, or the increase in bioactive antioxidant compounds (i.e. isoflavones) found within soybean meal. The latter has yielded mixed results in PRRSV infected pigs (Greiner et al., 2000; Smith et al., 2019).

Furthermore, based on previous work from our group we determined that the potential benefits of feeding increased soybean meal during a PRRSV challenge is likely not related to digestibility of nutrients or AA (Schweer et al., 2018b). Additionally, basal endogenous losses of AA were only nominally different in PRRSV challenged pigs compared to healthy control pigs and this translated to minimal differences in standardized ileal digestibility (**SID**) of most AA (Schweer et al., 2018b). To further examine the impact of soybean meal, we have also studied how the relationship of Lys to energy impacts health-challenged pig performance. Using break

point analysis our group has reported that increasing SID Lys:ME to 110% to 120% above the NRC (2012) requirement resulted in improved growth performance and feed efficiency in grower pigs subjected to a PRRSV challenge, while unchallenged pigs did not benefit from a higher plane of AA (Schweer et al., 2018a). The increased Lys:ME ratio was achieved primarily by intact protein sources, while synthetic AA levels remained relatively constant. Reduction in feed intake during a disease challenge reduces nutrient availability to tissues, thus being the primary cause of reduced lean tissue accretion observed during a viral challenge (Helm et al., 2019). Therefore, we hypothesized that decreasing dietary energy concentrations may be beneficial during immune stimulation to help mitigate anorexia (i.e. improve feed intake). Moreover, it is unclear if the improved growth performance during a PRRSV challenge is attributed to increases in dietary SID AA (increase in CP), or if reducing ME to achieve the same ratio, thereby promoting feed intake, would yield similar results.

Therefore, the objective of this study was to evaluate the effects of increasing SID Lys:ME in PRRSV vaccinated and nonvaccinated pigs facing a subsequent PRRSV challenge on growth performance. Furthermore, we hypothesized that irrespective of how an increase in the SID Lys:ME (i.e. 120%) is achieved, by either an increase in g SID Lys or a reduction in ME would result in increased growth performance in PRRSV infected pigs compared to that of pigs fed a 100% SID Lys:ME diet. Lastly, we hypothesized that health-challenged pigs would exhibit the ability to eat to their energy needs.

Materials and Methods

All procedures adhered to the ethical and humane use of animals for research and were approved by the Iowa State University Institutional Animal Care and Use Committee (**IACUC**# 18-158). This study was conducted from September 2018 to March 2019 in Ames, IA.

Animal Housing and Experimental Design

Four hundred non-vaccinated, mixed sex (purebred Duroc sires by commercial Yorkshire-Landrace F1 females; 5.4 ± 1.23 kg BW), 19-21 d old weaned PRRS-naïve pigs were randomly selected from a single source sow farm and transported to Ames, IA. Upon arrival, all weaned pigs were randomly split by litter across two barns with identical configuration (i.e. ventilation, temperature set points, pen configuration, feeders and waterers). Each barn had 24 pens, however only 12 pens in each barn were utilized for the nursery acclimation phase and each pen was double stocked to contain 15-17 pigs. All pens were identical in size (3.66 m x 2.44 m), with fully slatted concrete flooring and two water cups. Each barn was climate controlled to thermoneutral conditions with propane heaters and wall ventilation fans which were adjusted accordingly as pig age increased. On day one post-placement, one barn was vaccinated intramuscularly with 1 mL of a modified live PRRS vaccine (Ingelvac PRRS® MLV, Boehringer Ingelheim, St. Joseph, MO), while the other barn was not PRRSV vaccinated. Throughout the 42 d nursery acclimation period all pigs were fed identical diets in three dietary phases, and all diets met or exceeded the nutritional requirements of the pig (NRC, 2012).

On d 42 post-weaning (25.6 ± 4.31 kg BW) pig numbers were reduced in all nursery pens to carry out the experimental phase during the grower period. This was achieved by randomly selecting 7-10 pigs within pen and barn (vaccine status) and placing them into clean, unused pens within the same barn. The grower phase of the study was carried out using 48

identical pens (3.66 m x 2.44 m wide, with fully slatted floors), containing a double sided 36 cm feeder and two nipple waters. Within vaccine status there were 24 pens in which all pigs received a common corn-soybean meal-based grower diet that met or exceeded the nutritional requirement (NRC, 2012) for weight range of pigs up until 14 d prior to PRRSV inoculation.

After a 14 d acclimation period (d 56 post-weaning) to the grower pens, all pigs in both barns (vaccinated 35.2 ± 0.60 kg BW; non-vaccinated 35.2 ± 0.65 kg BW) were randomly allotted to one of three dietary treatments with 8 pens per treatment per vaccine status. The three treatments per vaccine status were: 1) control, a diet formulated to contain 2.69 g SID Lys:ME (control diet representing 100% Lys:ME based on NRC 2012); 2) high Lys (**HL**), a diet containing 3.23 g SID Lys:ME achieved via increased inclusion of soybean meal and synthetic AA (120% ratio from control); and 3) low energy (**LE**), a diet containing 3.22 g SID Lys:ME achieved by reducing dietary ME via the inclusion of 18% fine grade, washed and dried sand (120% ratio from control). The three diets (Table 2.1) were formulated to contain 2.69, 3.23, and 3.22 g SID Lys:ME, representing 100, 120, and 120% of requirements for 35-75 kg BW pigs. This SID Lys:ME requirement was based on breakpoint analysis from the Schweer, et al., (2018) projections for 35-75 kg BW pigs, adjusted for NRC (2012) and Maschhoffs verified internal nutrient requirements. The three diets were meal form and formulated to meet or exceed NRC (2012) nutrient and energy requirements, and contained similar total calcium, available phosphorus and ratios of SID Thr, Trp, Met, Ile, and Val to SID Lys to avoid secondary AA deficiencies (Table 2.1).

On d 56 post-weaning, corresponding with day post inoculation (**dpi**) 0, all pigs in both barns were inoculated intramuscularly with 1 mL of a live field strain of PRRSV (1-18-4), containing 10^6 genomic PRRSV units per mL. For the next 42 dpi, pig BW, pen feed intake and

feed efficiency were collected and calculated weekly on dpi 0, 7, 14, 21, 28, 35, and 42. Pigs were allowed unrestricted access to feed and water throughout the 42 d PRRSV challenge. In addition, deceased pigs from the LE dietary treatment were gross necropsied to determine if sand had caused any irritation to the digestive tract. There was no gross visible evidence of sand induced irregularities of gastrointestinal tracts in these pigs.

Diet analysis

The three experimental diets used during the PRRSV challenge were analyzed for energy and nutrient composition. Analysis of dietary gross energy (**GE**) content was determined using bomb calorimetry (Oxygen Bomb Calorimeter 6200, Parr Instruments, Moline, IL). Diet samples were analyzed for dietary dry matter (**DM**) using method 934.01 according to AOAC (2007). Dietary AA and nitrogen (**N**) analysis were conducted by University of Missouri Experimental Station Chemical Laboratories (Columbia, MO). Amino acid and N analysis were performed using method 994.12, 999.13, and 990.03 according to AOAC (2007) methods, and CP was calculated (N × 6.25).

Blood collection and analysis

Two pigs in each pen were randomly selected and these same two pigs were snare-restrained and serial bled on dpi -7, 0, 7, 14, 21, 28, 35 and 42. Blood samples (8-10 mL) were collected from the jugular vein into serum tubes (BD Vacutainer, Franklin Lakes, NJ) for routine diagnostic testing. Blood samples from pigs at 0 dpi were collected immediately before inoculation. All blood samples were allowed to clot, then serum separated by centrifugation (2,000 × *g*, 15 min at 4°C) pooled within dietary treatment and vaccine status, and stored at -80˚C until analysis. Serum aliquots were submitted to the Iowa State University Veterinary Diagnostic Laboratory (**ISUVDL**), Ames, IA for testing. Real-time polymerase chain reaction

(**RT-PCR**) and serum antibody testing for PRRSV were performed using commercial reagents (VetMAX™ NA and EU PRRSV real-time-PCR, Thermo Fisher Scientific, Waltham, MA) and a commercial ELISA kit (HerdCheck® PRRS X3, IDEXX Laboratories, Inc., Westbrook, ME), respectively. A serum viremia cycle threshold (**Ct**) ≥ 37 was considered negative and serology antibody was considered negative when sample-to-positive (**S:P**) ≤ 0.40.

Statistical analysis

Within vaccine status and with pen considered the experimental unit, all data were analyzed using a complete randomized design with the PROC MIXED procedure of Statistical Analysis System (**SAS**) 9.4 (SAS Inst. Inc., Cary, NC). All performance data were analyzed for the fixed effects of dietary treatment consisting of control, HL and LE Lys:ME, representing 2.69, 3.23 and 3.22 g SID Lys:ME, respectively. Least-squares (**LS**) means were determined for each treatment using the LS means statement and differences in LS means were produced using the pdiff option. Tukey's multiple comparison adjustment was used on each LS mean pairwise comparison. Data were reported as LS means and standard error of the mean. Differences were considered significant when $P < 0.05$ and a tendency when $0.05 < P < 0.10$.

Results

Diet Analysis

During the PRRSV challenge period, the experimental diets were formulated to contain 2.69, 3.23 and 3.22 g SID Lys per Mcal ME (Table 2.1). Proximate and AA analysis of the diets were conducted to verify that the diets were formulated similar to the predicted values (Table 2.1). Analyzed GE of the diets were 3.87, 3.86 and 3.01 Mcal/kg, representing the control, HL

and LE dietary treatments, respectively. These results confirmed the formulated 20% reduction in dietary energy LE in comparison to the control and HL diets.

Population vaccine status, health and response to PRRSV

Serum samples were pooled within dietary treatment and vaccine status to confirm weekly PRRSV viremia and antibody titers (dpi 0-42). The serology responses to the PRRS vaccine and the PRRSV challenge are reported in Table 2.1. Prior to PRRSV inoculation, PRRSV viremia was not detected in pigs irrespective of vaccine status based on serum Ct values ≥ 37. As expected, the PRRSV vaccinated pigs had detectable PRRSV antibodies 56 days post-vaccination, while the non-vaccinated pigs were deemed negative for PRRSV antibodies with S:P ≤ 0.40. The success of the PRRSV challenge was confirmed via PCR over the 42 d challenge period. By 7 dpi, irrespective of diet and vaccination status, PRRS viremia Ct values were reported in the range of 16 to 26 (considered positive if < 37; Table 2.2). As expected, PRRSV Ct values increased (i.e. viremia decreased) as pigs seroconverted. Vaccinated pigs had detectable PRRSV antibodies (S:P ratio) prior to PRRSV inoculation, and PRRSV antibody levels increased throughout the challenge period and plateaued at 28 dpi, at which time all vaccinated pigs were considered non-viremic (Ct > 37; Table 2.2). As expected, nonvaccinated pigs experienced a longer duration and magnitude of PRRSV viremia based on diagnostics. Following PRRSV inoculation, antibody titers for nonvaccinated pigs increased throughout the challenge period (Table 2.2).

Diagnostic testing also indicated that all pigs, irrespective of PRRS vaccination status, became naturally infected with Porcine Circovirus 2 (**PCV2**) between dpi 7 and 14, as confirmed by PCR; all pigs had not received PCV2 vaccinations prior to this experiment. As a result of this PRRSV and PCV2 co-infection, the PRRSV vaccinated and non-vaccinated barns experienced

11 and 22 mortalities, respectively, equating to 5.6 and 11.3% mortality over the test period. However, mortality was not different across dietary treatment (data not shown). A common cause of mortality, as reported by necropsy and diagnostics via the ISUVDL, was attributed to systemic effects of PRRSV and PCV2, with *Streptococcus suis* sepsis resulting in rapid death. Due to the severity of disease from unintended PCV2 infection, intentional PRRSV challenge, and secondary bacterial infections, all pigs were placed on water amoxicillin (Vet Rx Pharmacy, St. Peter, MN) from 14 to 21 dpi to decrease the impact of opportunistic secondary bacterial pathogens. From 22 to 30 dpi, all pigs received sodium salicylate (Aurora Pharmaceutical LLC., Northfield, MN) through the water with a daily target dose of 50 mg/kg body weight to help mitigate any febrile response associated with the multifactorial infection.

Performance: PRRSV vaccinated pigs

Prior to the disease challenge period (dpi 0) all pigs were fed a common nursery diet and no differences in pig performance parameters within the vaccinated pens were detected ($P > 0.10$; Table 2.3). From 0 to 7 dpi, there was a tendency ($P = 0.071$) for average daily gain (**ADG**) to be increased by 150% in the HL pigs compared to the control treatment, while LE was not different from either treatment ($P > 0.05$). Growth rates were similar between treatments for all other weekly weigh periods ($P > 0.10$; 7 to 42 dpi). An increase ($P < 0.05$) in average daily feed intake (**ADFI**) was observed weekly throughout the challenge period, with the exception of dpi 14 to 21 in which a tendency for ADFI was observed ($P < 0.10$) as a result of the LE treatment compared to the control and HL dietary treatments. From 28 to 35 dpi, feed efficiency (gain-to-feed, **G:F**) was greatest for pigs fed the HL dietary treatment, lowest for pigs fed the LE treatment, and intermediate for those fed control diet; however, G:F differences were not detected in any other weekly growth periods ($P > 0.05$).

For the overall challenge period (Table 2.5), increasing SID Lys:ME to 120% of NRC (2012) requirement during the 42 d PRRSV challenge period increased ADG ($P < 0.01$), regardless of how the 120% ratio was achieved by either increasing g SID Lys (HL) or decreasing ME (LE). Overall ADFI increased 19.8% as a result of LE dietary treatment compared to control ($P < 0.01$), whereas the HL treatment was similar to the control. When expressing overall ADFI on a ME intake per day, the HL pigs had significantly higher ME intakes compared to the LE ($P < 0.05$), with the control pigs being intermediate (Table 2.5). An increase in overall G:F was observed in pigs fed the HL treatment compared to pigs fed the control and LE treatments ($P < 0.01$), which were not different from each other. End BW of pigs fed HL and LE treatments were improved 6.9 kg and 4.2 kg, respectively, in comparison to the control ($P < 0.05$).

Performance: PRRSV non-vaccinated pigs

In the non-vaccinated pigs, prior to the disease challenge period (dpi 0) there were no differences in pig performance parameters ($P > 0.10$; Table 2.4). Throughout the challenge period, pigs remained PRRSV seropositive until 42 dpi (Table 2.2), confirming PRRSV inoculation was successful. Weekly growth performance results are shown in Table 2.4. From 0 to 7, 21 to 28, and 28 to 35 dpi ADG increased in pigs fed the HL and LE dietary treatments relative to control ($P < 0.05$), with no differences between treatment during the other weekly weigh periods. There were no differences ($P > 0.05$) in ADFI between treatments during the first 4 weekly weigh periods. An increase in ADFI was observed from 28 to 35 and 35 to 42 dpi as an effect of LE dietary treatment ($P < 0.01$). From 0 to 7, 21 to 28, and 28 to 35 dpi, G:F was increased in pigs fed the HL and LE diets compared to control ($P < 0.05$); with no other G:F differences observed between treatments throughout other weekly growth periods.

Overall growth performance results are shown in Table 2.5. Overall, increasing SID Lys:ME to 120% of NRC (2012) requirement during the 42 d PRRSV challenge period increased ADG ($P < 0.05$), regardless of how the 120% ratio was achieved by either increasing g SID Lys or decreasing ME. Overall ADFI increased 16.6% as a result of LE dietary treatment with respect to control ($P < 0.01$); with no difference seen between HL and control ($P > 0.05$). Further, during the overall challenge period daily ME intake (Mcal/d) tended ($P = 0.077$) to differ, with the LE pigs having the lowest ME intake per day compared to the control and HL pigs (Table 2.5). Dietary treatment had no effect on overall G:F ($P > 0.10$). End BW of pigs fed HL and LE treatments were improved 5.4 and 5.2 kg, respectively, in comparison to control ($P < 0.05$).

Discussion

It is well established that Lys is the first-limiting AA in healthy pigs, and to ensure that the targeted amount of Lys is being consumed by the pig, diets are formulated on a ratio of Lys to energy (i.e. g SID Lys:ME). Previous breakpoint analysis from our group (Schweer et al., 2018a) has reported that during both an experimental and natural PRRSV challenge, increasing SID Lys:ME 10% to 20% above NRC (2012) requirements, resulted in improved growth performance and feed efficiency. This increase in Lys:ME is presumably accounting for the reduced feed and Lys intake (Schweer et al., 2017) thus preserving lean tissue. When formulating to 100% of NRC requirement in PRRSV challenge pigs, Lys intake would be reduced, which is thought to contribute to a depleted AA pool which likely results in a reduction of lean tissue accretion (Helm et al., 2019). Therefore, our objective herein was to confirm the performance benefit of increasing the dietary SID Lys:ME in PRRSV vaccinated and non-

vaccinated grower pigs experiencing a PRRSV challenge. Furthermore, we hypothesized that irrespective of how the 120% SID Lys to ME ratio was achieved via diet formulation, either by increasing Lys or reducing ME, it would result in increased growth performance in PRRSV infected pigs compared to the NRC (2012) recommended Lys:ME requirement.

It is inevitable throughout the swine industry that growing pigs will experience a performance-impacting disease challenge. A PRRSV challenge is shown to attenuate growth rates 30 to 59% compared to healthy controls (Che et al., 2011; Rochell et al., 2015; Schweer et al., 2016). The differences in severity of this negative impact on growth performance is thought to be a result of pig age, viral strain and PRRS viral clearance rates (Murtaugh et al., 2002). In recent years, Rochell et al. (2015) and Schweer et al., (2018a) have reported that dietary treatment can aid in improving growth performance and feed efficiency of pigs experiencing PRRSV challenge. In particular, Schweer et al. (2018a) reported that increasing the dietary SID Lys:ME by 10 to 20% above NRC (2012) requirement in 25-50 kg pigs increased growth performance and feed efficiency. However, it is unclear if the improved growth performance during this PRRSV challenge was attributed to increase in SID AA, CP or other functional factors associated with soybean meal.

In this research, due to the intentional formulation of the diets, CP levels remained similar in both the control and LE diets, along with relatively similar soybean meal inclusion levels of 19.35% and 21.95% respectively. However, the HL diet was formulated to have an increased CP level with the increased inclusion of soybean meal (26.5%) in comparison to control and LE diets. Soybean meal contains naturally occurring bioactive components, i.e. isoflavones, that have antiviral activity in PRRSV challenged pigs (Greiner et al., 2001); however no differences in viremia (i.e. PCR Ct values) or antibody titers were observed due to

dietary treatment in this study. In a study feeding diets with a high and low soybean meal inclusion level to newly weaned pigs, pigs fed high soybean meal diets had reduced immune stress and increased ADG during a PRRSV challenge (Rochell et al., 2015). When utilizing soybean meal to increased Lys:ME ratio, various other essential and non-essential AA are likely also increasing in the diet which may be beneficial. It has been shown that during a LPS challenge pigs fed increased levels of Met and Met+Cys resulted in increased protein deposition, indicating that the optimal Met:Met+Cys is greater during immune system stimulation (Litvak et al., 2013). Additionally, Thr and Trp are two AA that play an important role in the immunity of animals (Li et al., 2007). Threonine is a major component of plasma immunoglobulin G (IgG) and has shown to enhance antibody production and serum IgG levels in young pigs challenged with *Escherichia coli* (Wang et al., 2006). Additionally, Trp is a precursor of serotonin (5-hydroxytryptamine) and feed intake regulation. Limited research has been conducted to evaluate the effects of altering dietary Thr and Trp during immune challenge. However, when evaluating the effects of Thr and Trp supplementation on attenuation of immunological challenge-induced growth reduction in PRRS vaccinated pigs, Xu et al. (2014) reported increased feed intake and improved ADG in Thr and Trp supplemented pigs compared to control after PRRS vaccination. Altogether, increasing soybean meal inclusion in the diet likely increases the intake of multiple AA, not just Lys, thus reducing the need for lean tissue catabolism and preserving lean tissue during a disease challenge.

To further test the benefit of increasing the Lys:ME of PRRSV challenged pigs, 3.22 g SID Lys:ME was also achieved via a dilution of energy (LE dietary treatment), as discussed previously. This LE diet resulted in increased ADG compared to the control diet and resulted in similar ADG to the HL treatment. Although increased CP and AA may be beneficial, these data

indicated that the Lys:ME is critical to driving the improved performance responses in a PRRSV challenged pig. By default, the 20% reduced ME diet (LE) also indicates that viral challenged pigs were able to adjust their voluntary feed intake to eat to their energy needs. The theory of pigs eating to their energy needs implies that a dilution of dietary energy would result in an increase in feed intake. Reduction in ADFI in newly weaned pigs has been reported as a result of increased energy concentration in the diet in both healthy and immune-challenged pigs when compared to diets with lower energy concentration (van Heugten et al., 1996; Oresanya et al., 2007). In *Escherichia coli* LPS challenged nursery pigs, feed intake was reduced in pigs fed high energy diets; however, energy intake was equal between high and low energy diets, indicating immune stimulated pigs have the ability to adjust their voluntary feed intake to meet their energy needs regardless of dietary energy concentration (van Heugten et al., 1996). In the current study, we report that a 20% reduction in dietary ME increased ADFI 20% and 17% in PRRS vaccinated and non-vaccinated pigs, respectively, in the face of a PRRSV challenge. These results are in agreement with a previous dilution study conducted by Baker et al. (1968) in which 53 kg pigs fed a diet with 20% inclusion of sand resulted in a 20% increase in ADFI, in non-disease challenged pigs. Collectively, these results indicate the pig's ability to adjust their voluntary feed intake to achieve a level of energy needs in both healthy and immune-challenged situations. Thus, increasing dietary energy concentrations would likely result in a reduction in feed intake to maintain a constant daily energy intake.

Although the highest ADG in vaccinated (3.23 g SID Lys:ME) and non-vaccinated (3.22 g SID Lys:ME) pigs did not result from the same dietary treatment, growth was increased the greatest at a similar total Lys intake of 24.1 g/d in both PRRSV vaccinated and non-vaccinated pig The NRC (2012) recommends a Lys intake of 16.9 g/d in 35-75 kg pigs; however, two diets

in the current study were formulated to 120% of NRC requirement for the disease challenge period which equated to ~20.3 g Lys/d. Although growth rate and PRRSV status of the pigs differ, these results are similar to Schweer et al. (2018a) in which growth was optimized at similar total daily Lys intake in control and PRRSV infected pigs. Interestingly the results from the current study and Schweer et al. (2018a) differ from previous work reporting that Lys requirement (g/day basis) is reduced in immune stimulated pigs compared to non-immune stimulated pigs (Williams et al., 1997; Zimmerman et al., 1997). This is thought to be attributed to increased lean tissue deposition in pigs with low immune system activity compared to those with high immune stimulation. Nonetheless, the results from this study support the theory that in the event of a stressor such as a disease challenge, AA requirements may change due to increased metabolic activity and the repartitioning of nutrients away from lean tissue accretion (i.e. protein catabolism). Thus, indicating the importance and impact of feed intake during a disease challenge. Overall, by decreasing ME in the diet to achieve 120% of NRC (2012) SID Lys:ME requirement we were able to increase ADFI attenuating a portion of the growth depression commonly observed during a PRRSV challenge.

In today's swine industry, PRRS vaccination strategies are commonly implemented to serve as a line of protection in the event of a PRRSV challenge, however available vaccines have varying efficacy (Osorio et al., 1998 ; Mavromatis et al., 1999; Meng, 2000). The efficacy of PRRSV vaccines is commonly assessed by evaluating the vaccines' ability to reduce viremia after the challenge, which is crucial for mitigating the negative effects associated with PRRSV. In young, naive pigs it is often a concern that early vaccination is ineffective due to the immature immune system's inability to effectively respond and build immunity. However, a study conducted by Jeong et al. (2018) concluded that PRRS MLV vaccination of pigs as early as day

one, and as late as day 182 of age, resulted in improved growth performance in the face of a natural PRRSV challenge. Although not the object of paper, the PRRSV vaccinated group had reduced mortality and improved growth performance compared to non-vaccinated pigs throughout the 42 d challenge period; however, PCV2 likely had a major impact on mortality in this study. These findings are in agreement with previous findings (Park et al., 2014; Jeong et al., 2018; Oh et al., 2019).

In summary, this work validates that during a controlled PRRSV challenge (also naturally co-challenged with PCV2), increasing SID Lys:ME to 120% in grower pigs aids in the mitigation of negative growth performance associated with mixed infections including PRRSV challenge (Schweer et al., 2018a). Irrespective of vaccination status, a 20% dilution of energy in the diet resulted in increased feed intake, translating to an increase in ADG and end BW in comparison to a control throughout a PRRSV challenge. The results from this study support the theory that in the event of a disease challenge, AA requirements may change due to increased metabolic activity and the repartitioning of nutrients away from lean tissue accretion, indicating the importance and impact that feed intake has during a disease challenge. Feed efficiency was most improved as a result of the HL dietary treatment, suggesting that from a feed efficiency standpoint, increasing SID Lys was the most beneficial mitigation strategy rather than diluting ME. However, in non-vaccinated pigs both the HL and LE treatment resulted in comparable increases in ADG and end BW, suggesting that during a severe health-challenge reducing dietary energy is also an effective strategy to achieve a 120% SID Lys:ME. The utilization of sand to dilute dietary energy is not a practical approach. However, the utilization of dietary fiber to dilute energy could be a more practical industry approach. Overall, increasing SID Lys:ME 20% above the recommended NRC (2012) requirement in PRRSV infected pigs resulted in increased

growth performance in comparison to control. This performance was observed irrespective of vaccination status or the dietary strategy used to achieve the 120% SID Lys:ME.

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Table 2.1. Experimental diet composition, as fed basis, 35 to 70 kg BW pig

Ingredients, %	g SID[1] Lys:Mcal ME		
	2.69 (control)	**3.23 (HL)**	**3.22 (LE)**
Corn	75.91	68.89	56.22
Soybean meal (48% CP)	19.35	26.46	21.95
Limestone	0.94	0.93	0.84
Monocalcium phosphate (21%)	0.74	0.60	0.90
Salt	0.46	0.46	0.47
Sand	-	-	18.00
Fat, Animal-Vegetable Blend	1.68	1.62	0.84
L-Lysine Sulfate (54.6%)	0.52	0.55	0.41
L-Threonine	0.11	0.12	0.09
DL-Methionine	0.11	0.16	0.12
L-Valine	0.02	0.03	0.01
Vitamin Premix[2]	0.03	0.03	0.03
Trace Mineral Premix[3]	0.08	0.08	0.08
Copper sulfate (25.2%)	0.06	0.06	0.06
Phytase 500FTU/KG	0.01	0.02	0.00
Calculated composition			
DM, %	86.28	85.45	88.88
CP, %	14.77	17.60	14.48
ME, Mcal/kg	3.31	3.31	2.67
NE, Mcal/kg	2.58	2.54	2.04
Total Calcium, %	0.58	0.58	0.58
P, Available %	0.24	0.24	0.24
Lys, Total %	0.99	1.18	0.96
SID AA			
Lys	0.89	1.07	0.86
Thr:Lys	0.61	0.61	0.61
Met+Cys:Lys	0.57	0.57	0.57
Trp:Lys	0.16	0.17	0.18
Ile:Lys	0.56	0.58	0.59
Val:Lys	0.65	0.65	0.65
SID Lys:ME, g/Mcal	2.69	3.23	3.22
Analyzed composition			
DM, %	87.03	87.06	87.05
CP, %	14.29	16.74	17.05
GE, Mcal/kg	3.87	3.86	3.08
Lys, Total %	0.77	1.22	1.08
Total AA:Lys			
Thr:Lys	0.86	0.56	0.53
Met+Cys:Lys	0.78	0.56	0.61
Ile:Lys	0.81	0.58	0.58
Val:Lys	0.88	0.65	0.64

[1]SID = standardized ileal digestibility

[2]Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 5,291 IU as vitamin A acetate; vitamin D3, 827 IU as vitamin D-activated animal sterol; vitamin E, 26 IU as α-tocopherol acetate; menadione, 1.5 mg as menadione dimethylpyrimidinol bisulfite; vitamin B12, 0.02 mg; riboflavin, 6.0 mg; pantothenic acid, 22 mg as calcium pantothenate; niacin, 30 mg.

[3]Provided the following quantities of trace minerals per kilogram of complete diet: Fe, 124 mg as iron sulfate; Zn, 124 mg as zinc oxide; Mn, 29 mg as manganese sulfate; Cu, 12 mg as copper sulfate; I, 0.22 mg as calcium iodate; and Se, 0.22 mg as sodium selenite.

Table 2.2. Overall effects of increasing the ratio of standardized ileal digestible (SID) lysine and reduced metabolizable energy (ME) on PRRSV viremia and antibody titers in PRRSV challenged pigs

Parameter[2]	g SID[1] Lys:Mcal ME					
	Vaccinated			Nonvaccinated		
	2.69 (control)	3.23 (HL)	3.22 (LE)	2.69 (control)	3.23 (HL)	3.22 (LE)
PRRSV Ct value[3]						
dpi 0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0
dpi 7	25.8	25.3	24.1	17.6	16.5	19.6
dpi 14	32.0	26.8	32.1	25.4	25.3	26.2
dpi 21	35.4	35.6	≥37.0	27.3	20.1	26.8
dpi 28	≥37.0	≥37.0	≥37.0	31.0	30.1	29.8
dpi 42	≥37.0	≥37.0	≥37.0	≥37.0	36.7	≥37.0
PRRSV S/P ratio[4]						
dpi 0	2.025	1.890	1.881	-0.006	-0.008	-0.005
dpi 7	2.005	1.773	1.949	0.304	0.154	0.220
dpi 14	2.011	1.943	1.995	1.266	1.158	1.307
dpi 21	1.919	2.016	1.941	1.380	1.217	1.181
dpi 28	2.185	2.049	1.859	1.273	1.242	1.279
dpi 42	1.978	1.894	1.940	1.685	1.285	1.571

[1]SID = standardized ileal digestibility
[2]Pooled serology within treatment and vaccine status
[3]Cycle threshold (Ct), Ct ≥ 37.0 denotes PRRS negative.
[4]PRRSX3 antibody sample to positive (S/P) ratio, ≤ 0.40 denotes PRRS negative.

Table 2.3. Effects of increasing the ratio of standardized ileal digestible (SID) lysine to metabolizable energy (ME) on growth performance in PRRSV challenged, vaccinated growing pigs

	g SID[1] Lys:Mcal ME[4]				
Parameter	**2.69 (control)**	**3.23 (HL)**	**3.22 (LE)**	**SEM**	***P*-Value**
Nursery[2]					
Start BW, kg	5.5	5.4	5.3	0.115	0.318
ADG, kg	0.482	0.490	0.478	0.017	0.883
ADFI, kg	0.755	0.798	0.760	0.018	0.277
G:F	0.720	0.708	0.709	0.022	0.911
End BW, kg	25.7	25.9	25.1	0.647	0.651
PRRSV Challenge[3]					
dpi 0 to 7					
ADG, kg	0.416	0.633	0.511	0.062	0.071
ADFI, kg	1.120[b]	1.411[a]	1.324[ab]	0.063	0.014
G:F	0.375	0.452	0.396	0.050	0.534
End BW, kg	37.4[b]	40.6[a]	38.3[b]	0.532	0.002
dpi 7 to 14					
ADG, kg	0.407	0.506	0.520	0.087	0.615
ADFI, kg	1.221[b]	1.462[ab]	1.494[a]	0.073	0.033
G:F	0.327	0.336	0.344	0.061	0.980
End BW, kg	40.6[b]	44.1[a]	42.0[ab]	0.809	0.021
dpi 14 to 21					
ADG, kg	0.790	0.966	0.949	0.092	0.348
ADFI, kg	1.729	1.745	2.027	0.090	0.052
G:F	0.458	0.536	0.467	0.041	0.355
End BW, kg	45.8[b]	50.8[a]	48.6[b]	0.857	0.002
dpi 21 to 28					
ADG, kg	0.968	1.016	1.090	0.092	0.647
ADFI, kg	2.102[b]	2.221[ab]	2.525[a]	0.092	0.013
G:F	0.474	0.459	0.445	0.036	0.846
End BW, kg	52. 7[c]	58.6[a]	56.6[b]	0.937	0.001
dpi 28 to 35					
ADG, kg	0.912	1.045	0.967	0.072	0.434
ADFI, kg	2.398[c]	2.438[b]	2.792[a]	0.078	0.004
G:F	0.376[b]	0.430[a]	0.346[c]	0.021	0.035
End BW, kg	59.5[b]	66.0[a]	63.3[a]	1.037	0.001
dpi 35 to 42					
ADG, kg	0.873	1.073	1.070	0.083	0.178
ADFI, kg	2.456[c]	2.590[b]	3.053[a]	0.068	<.0001
G:F	0.354	0.415	0.350	0.026	0.181
End BW, kg	66.6[b]	73.5[a]	70.8[a]	1.194	0.003

[1]SID = standardized ileal digestibility

[2]Nursery period (-56 to -14 dpi), all pigs fed common diet; n = 4 pens/treatment and 15 to 17 pigs/pen

[3]Challenge period (0 to 42 dpi), all pigs fed experimental diets; n=8 pens/treatment and 7 to 10 pigs/pen

[4]Means without a common superscript (a-c) differ significantly ($P < 0.05$)

Table 2.4. Effects of increasing the ratio of standardized ileal digestible (SID) lysine to metabolizable energy (ME) on growth performance in PRRSV challenged, non-vaccinated growing pigs.

	g SID[1] Lys:Mcal ME[4]				
Parameter	**2.69 (control)**	**3.23 (HL)**	**3.22 (LE)**	**SEM**	***P*-Value**
Nursery[2]					
Start BW, kg	5.3	5.3	5.5	0.245	0.777
ADG, kg	0.478	0.472	0.488	0.009	0.506
ADFI, kg	0.749	0.743	0.777	0.013	0.201
G:F	0.774	0.730	0.731	0.025	0.431
End BW, kg	25.4	25.1	26.1	0.487	0.350
PRRSV Challenge[3]					
dpi 0 to 7					
ADG, kg	-0.022^{b}	0.119^{ab}	0.275^{a}	0.064	0.014
ADFI, kg	0.839	0.879	1.001	0.052	0.083
G:F	-0.011^{b}	0.121^{ab}	0.270^{a}	0.070	0.034
End BW, kg	35.2	36.9	36.0	0.663	0.228
dpi 7 to 14					
ADG, kg	0.265	0.319	0.340	0.061	0.669
ADFI, kg	0.826	0.804	0.938	0.052	0.183
G:F	0.342	0.385	0.369	0.066	0.898
End BW, kg	37.0	39.1	38.3	0.821	0.232
dpi 14 to 21					
ADG, kg	0.759	0.667	0.617	0.094	0.569
ADFI, kg	1.412	1.463	1.587	0.069	0.209
G:F	0.528	0.451	0.390	0.050	0.180
End BW, kg	42.9	43.8	42.6	1.156	0.766
dpi 21 to 28					
ADG, kg	0.587^{b}	0.782^{ab}	0.894^{a}	0.069	0.017
ADFI, kg	1.848	1.872	2.130	0.093	0.087
G:F	0.317^{b}	0.414^{ab}	0.425^{a}	0.028	0.023
End BW, kg	47.2	50.1	49.3	1.306	0.302
dpi 28 to 35					
ADG, kg	0.842^{b}	1.086^{a}	0.937^{ab}	0.058	0.025
ADFI, kg	2.153^{b}	2.283^{b}	2.551^{a}	0.045	<.001
G:F	0.392^{b}	0.477^{a}	0.366^{b}	0.026	0.018
End BW, kg	53.1^{b}	57.8^{a}	55.7^{b}	1.212	0.041
dpi 35 to 42					
ADG, kg	1.003	1.109	1.056	0.074	0.607
ADFI, kg	2.297^{b}	2.423^{ab}	2.724^{a}	0.087	0.009
G:F	0.439	0.454	0.388	0.023	0.139
End BW, kg	60.4^{b}	65.8^{a}	63.6^{b}	1.245	0.021

[1]SID = standardized ileal digestibility

[2]Nursery period (-56 to -14 dpi), all pigs fed common diet; n = 4 pens/treatment and 15 to 17 pigs/pen

[3]Challenge period (0 to 42 dpi), all pigs fed experimental diets; n=8 pens/treatment and 7 to 10 pigs/pen

[4]Means without a common superscript (a-c) differ significantly ($P < 0.05$)

Table 2.5. Overall effects of increasing the ratio of standardized ileal digestible (SID) lysine to metabolizable energy (ME) on growth performance in PRRSV challenged pigs.

	g SID[1] Lys:Mcal ME[2,4]				
Parameter	**2.69 (control)**	**3.23 (HL)**	**3.22 (LE)**	**SEM**	***P*-Value**
Vaccinated[3]					
Start BW, kg	34.7	36.1	34.7	0.600	0.178
End BW, kg	66.6[b]	73.5[a]	70.8[a]	1.194	0.003
ADG, kg	0.728[b]	0.873[a]	0.851[a]	0.033	0.013
ADFI, kg	1.838[b]	1.978[b]	2.202[a]	0.054	0.001
ME intake/d, Mcal	6.19[ab]	6.54[a]	5.88[b]	0.172	0.029
G:F	0.394[b]	0.438[a]	0.391[b]	0.010	0.005
Non-vaccinated[3]					
Start BW, kg	35.4	36.1	34.0	0.647	0.104
End BW, kg	60.4[b]	65.8[a]	65.6[b]	1.245	0.021
ADG, kg	0.572[b]	0.680[a]	0.687[a]	0.030	0.024
ADFI, kg	1.563[b]	1.621[b]	1.823[a]	0.047	0.003
ME intake/d, Mcal	5.17	5.37	4.87	0.139	0.077
G:F	0.334	0.384	0.368	0.014	0.135

[1]SID = standardized ileal digestibility
[2]n = 8 pens/treatment and 7 to 10 pigs/pen
[3]Overall challenge period (0 to 42 dpi), pigs fed experimental diets
[4]Means without a common superscript (a-c) differ significantly ($P < 0.05$)

CHAPTER 3. THE IMPACT OF INCREASED SID LYSINE TO METABOLIZABLE ENERGY RATIOS DURING A PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS AND *MYCOPLASMA HYOPNEUMONIAE* CHALLENGE IN GROW-FINSIH PIGS

A manuscript prepared for submission to the *Journal of Translational Animal Science*

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Abstract

Porcine reproductive and respiratory syndrome virus (**PRRSV**) and *Mycoplasma hyopneumoniae* (**MHP**) are major pathogens that antagonize growth performance in growing pigs. Thus, possible nutritional strategies to help mitigate the effects of these health-challenges are of interest. Therefore, the objective of this study was to evaluate whether increasing the dietary ratio of SID Lys to metabolizable energy (**ME**) 120% above the requirement of healthy pigs could enhance growth performance in grow-finish pigs facing an experimental PRRSV and MHP challenge. Vaccinated (**vac+**; mucosal killed autogenous PRRSV vaccine) and non-vaccinated (**vac-**; no PRRSV vaccine) grower pigs were subject to a PRRSV challenge (Exp. 1)

followed by a late finishing MHP challenge (Exp. 2). In Exp. 1, a total of 464 mixed sex pigs (PRRSV vaccinated 33.6 ± 1.44 kg BW; non-vaccinated 34.7 ± 1.43 kg BW) were allotted to one of three dietary treatments: 1) a control diet formulated to contain 2.98 g SID Lys:ME (representing 100% of requirement), a diet containing 3.57 g SID Lys:ME achieved by increasing Lys (120% of requirement, **HL**) and a diet containing 3.57 g SID Lys:ME achieved by a reduction in dietary energy via a fibrous feedstuff and increased Lys (120% of requirement, **HF**). Pigs were randomly allotted across two barns, each containing 24 pens with 9-10 pigs per pen (16 pens/diet and 24 pens/vaccine status). In Exp. 1, on day post inoculation (**dpi**) 0, all pigs were intranasally inoculated with live PRRSV and started on experimental diets. Weekly and overall challenge period pig performance were assessed. Overall, vaccination did not have an effect on overall average daily gain (**ADG**) and average daily feed intake (**ADFI**); however, a tendency for non-vaccinated pigs to have increased feed efficiency (gain-to-feed, **G:F**) compared to vaccinated pigs was observed ($P < 0.10$). A tendency was also observed for HL pigs to have the greatest ADG (0.878 kg), control pigs to be intermediate (0.856 kg) and HF pigs the lowest ADG (0.830; $P < 0.10$). Overall ADFI was increased 8.6% and 3.6% in HF and HL pigs respectively compared to control ($P < 0.05$). An increase in overall G:F was observed in pigs fed control and HL diet compared to HF, 3.3% and 11.2%, respectively ($P < 0.05$). At the conclusion of the 42 d PRRSV study, end BW did not differ between dietary treatment or vaccination status ($P > 0.05$). Eight days following the conclusion of the PRRSV challenge study, Exp. 2 began with a total of 464 mixed sex pigs (79.57. ± 8.97 kg BW) allotted to one of two dietary treatments (1.95 and 2.34 g SID Lys:ME, representing 100% and 120% of requirement, respectively) for a 40 d MHP challenge study. The same pen design was utilized from Exp. 1 with 9-10 pig per pen (12 pens/diet/MHP status). On MHP dpi 0, one barn was inoculated with

MHP, while the other barn was not inoculated (control), all pigs were started on experimental diets. No differences in overall ADG, ADFI, G:F or end BW due to dietary treatment in MHP non-challenged pigs ($P > 0.05$). The MHP challenged pigs also had no difference in overall ADG, ADFI, G:F or end BW in response to dietary treatment ($P > 0.05$). In summary, PRRSV challenged grower pigs had increased in ADFI when energy was diluted in the (HF) diet, compared to control pigs improving growth performance. Regardless of vaccination status, pigs fed 120% Lys:ME diets had slightly improved overall growth performance in response to a PRRSV challenge. In the event of a late finishing bacterial MHP challenge in MHP vaccinated pigs, increasing the Lys:ME had no effect on growth performance or end BW.

Keywords: lysine, metabolizable energy, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus

Introduction

Worldwide, bacterial and viral pathogens impact pig survivability and performance in all stages of swine production. In the U.S., two commonly reported respiratory pathogens that antagonize grow-finish pig performance include Porcine reproductive and respiratory virus (**PRRSV**) and *Mycoplasma hyopneumoniae* (**MHP**). In the case of PRRSV pathogen in the U.S., the disease of PRRS (Porcine reproductive and respiratory syndrome) is estimated to cost swine producers upwards of $644 million each year as it antagonized all stages of production causing increased morbidity, mortality and reduced growth in grow-finish pigs (Lunney et al., 2010; Holtkamp et al., 2013). *Mycoplasma hyopneumoniae* commonly causes enzootic pneumonia which is most frequently seen in grow-finish pigs. Highly variable degrees of disease (acute,

chronic or clinical) occur during a MHP challenge as secondary pathogens commonly arise, resulting in an intensified health-challenge (Tao et al., 2019). The primary concern associated with MHP is its ability to suppress the immune system by reducing mucociliary escalator clearance efficiencies, resulting in co-infections of secondary pathogens of both commensal bacteria and viruses (Thacker, 2001). In commercial conditions, pigs infected with MHP commonly have a prominent cough, but limited additional signs of disease present themselves. However, commonly pigs will have reduced growth rates resulting in increased market weight variation (Chase and Lunney, 2019; Pieter and Maes, 2019). The true economic impact of MHP is not well characterized due to the large variability between infected pigs/herds, in comparison to other bacterial and viral pathogens such as hemolytic *Escherichia coli* (***E. coli***) and PRRSV (Fairbrother et al., 2005; Holtkamp et al., 2013).

Although advances in diagnostics, vaccinations, animal management and biosecurity in swine production have been made, disease stressors such as PRRSV and MHP still have an impact on swine production today. Therefore, nutritional strategies to help mitigate these diseases are of interest. Nutritional requirements for healthy pigs have been well established (NRC, 2012); however, the nutrient requirements for pigs undergoing a health-challenge (viral or bacterial) have not been well defined, specifically amino acids (**AA**) requirements in relation to energy. In healthy pigs the first limiting AA when feeding a corn soybean meal-based diet is Lys. The dual role of AA in metabolism and protein synthesis along with the fact that protein synthesis is an energy demanding process, is the basis for a protein-energy interaction during growth (Moughan, 2018). Thus, in practical diet formulation AA are often expressed in relation to energy as a ratio (i.e. standardized ileal digestibility (**SID**) Lys to metabolizable (**ME**) (**SID Lys:ME**), ensuring a constant AA intake is achieved independent of dietary energy levels.

Previous work from our group utilizing breakpoint analysis has reported that increasing SID Lys:ME to 110% to 120% above requirement resulted in improved growth performance and feed efficiency in grower pigs subject to a PRRSV challenge, while unchallenged pigs did not benefit from an increased ratio (Schweer et al., 2018a). This concept has been further validated in PRRSV challenged pigs, in which Jasper et al. (2020) evaluated two formulation approaches to achieve a 120% Lys:ME. These two approaches included one diet having increased Lys via increased inclusion of soybean meal (**SBM**) and the second having a dilution of ME, via the inclusion of fine grade sand; both diets representing 120% of requirement. However, it is unknown if altering the Lys:ME utilizing dietary fiber to reduce energy improves performance of PRRSV challenged pigs or if a non-viral challenged pig will also respond in a similar manner. Therefore, the objective of this study was to further evaluate the formulation approach utilized to achieve a 120% Lys:ME, either by increasing SID Lys or a reducing ME in PRRSV challenged grower pigs. Additionally, we assessed if an increased Lys:ME is beneficial to growth performance of finishing pigs undergoing a non-viral challenge, such as MHP.

Materials and Methods

All procedures adhered to the ethical and humane use of animals for research and were approved by the Iowa State University Institutional Animal Care and Use Committee (**IACUC**# 18-158). Two experiments were conducted to evaluate the effects of increasing SID Lys:ME in PRRSV vaccinated and non-vaccinated pigs facing a subsequent PRSSV challenge from ~33 to 70 kg BW and during a late finishing HP challenge from ~80 to 130 kg BW. This study was conducted from June 2019 to November 2019 in Ames, IA.

Weaning, Vaccinations and Nursery Management

Four hundred and seventy-two mixed sex (purebred Duroc sires by commercial Yorkshire-Landrace F1 females; 5.6 ± 1.22 kg BW), ~21 d old weaned PRRS-naïve pigs were randomly selected from a single source sow farm and transported to Ames, IA. Prior to arrival, all pigs were intramuscularly vaccinated for Porcine circovirus 2 (PCV2) and MHP with 2 mL of Circumvent® PCV-M G2 vaccine (Merk Animal Health, Omaha, NE). Upon arrival, all pigs were randomly split across two barns with identical configuration (i.e. ventilation, temperature setpoints, pen configuration, feeders and waterers). Each barn contained 24 pens; however, only 12 pens in each barn were utilized for the nursery period as each pen was double stocked to contain 18 to 20 pigs. All pens were identical in size (3.55 m x 2.44 m), with fully slatted concrete flooring and two water cups.

Pigs were randomly allotted on the day of placement to one of three dietary treatments with 8 pens per dietary treatment. On day one and 23 post-placement, half of the pigs (6 pens/barn) were intranasally vaccinated with 2mL of Aptimmune Barricade Mucosal killed autogenous PRRSV vaccine (Dimond Animal Health, Des Moines, IA); while the rest of the pigs received no PRRSV vaccination (4 pens/treatment/vaccination status). Throughout the 42 d nursery period pigs were fed in three dietary phases (Table 3.1). Pens were allotted onto phase 1 diets consisting of three dietary treatments: low, medium, and high SBM (15, 25 and 35% SBM, respectively) fed for the first 12 days after weaning. In phase 2 (10 days) low, medium, and high SBM dietary treatments were increased to 25, 35 and 45% SBM, respectively. The third phase was a common nursery diet fed to all pigs for the remainder of the nursery period (20 days). All nursery diets met or exceeded nutritional requirements of this size pig (NRC, 2012) and represented the contrasting inclusion rates of SBM fed to wean pigs throughout the U.S. On day

eight post weaning, the pig population was confirmed positive for hemolytic *E. coli* and Rotavirus A and B via pooled fecal diagnostic testing at the Iowa State University Veterinary Diagnostic Lab (**ISUVDL**), Ames, IA. Individual pig BW and pen feed disappearance were recorded to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**) and gain-to-feed (**G:F**) at the end of each phase. Scour scores were recorded daily throughout phase one and two of the nursery; however, no scour score differences were reported across dietary treatments (data not shown). Throughout the nursery period, pigs were allowed unrestricted access to feed and water.

Experiment 1, PRRSV Challenge in 33 to 70 kg BW Pigs

On d 42 post-weaning (24.05 ± 4.33 kg BW) pig numbers were reduced in all nursery pens by randomly selecting 10 pigs within pen and barn, and placing them into clean, unused pen within the same barn. The grower PRRSV challenge phase of the study was carried out using 48 identical pens (3.66 m long x 2.44 m wide, with fully slatted floors), containing a double sided 36 cm feeder and two nipple waters. All pigs received a common corn-SBM-based grower diet that met or exceeded the nutritional requirements (NRC, 2012) for 14 d prior to PRRSV inoculation.

After the 14 d acclimation period (d 56 post-weaning) to the grower pens, 464 pigs (vaccinated 33.6 ± 1.44 kg BW; non-vaccinated 34.7 ± 1.43 kg BW) were randomly allotted to one of three dietary treatments with 8 pens/treatment/vaccine status with 9 to 10 pigs/pen. The three dietary treatments per vaccination status were: 1) control, a diet formulated to contain 2.98 g SID Lys:ME (representing 100% Lys:ME based on NRC 2012); 2) high Lys (**HL**), a diet containing 3.57 g SID Lys:ME achieved via increased inclusion of SBM (120% ratio from control) and 3) high fiber (**HF**), a diet containing 3.57 g SID Lys:ME achieved by reducing

dietary ME 8% via the inclusion of soy hulls in addition to increasing Lys via SBM (120% ratio from control). The three diets (Table 3.2) were formulated to contain 2.98, 3.57 and 3.57 g SID Lys:ME, representing 100, 120, and 120% of requirement for 34 to 70 kg BW pigs. The SID Lys:ME requirement was based on breakpoint analysis from (Schweer et al., 2018) projections, adjusted for NRC (2012) recommendations and validated internally by the Maschhoffs LLC. system (Carlyle, Illinois). The three diets were formulated to contain similar total calcium, available phosphorus and ratios of SID Thr, Trp, Met, Ile, and Val to SID Lys to avoid secondary AA deficiencies (Table 3.2).

On d 56 post-weaning, corresponding with day post inoculation (dpi) 0, all pigs in both barns were inoculated with a live field strain of PRRSV (1-18-4), administered with a single intranasal 2 mL dose of saline-diluted serum containing 10^6 genomic PRRSV units per mL. For the next 42 dpi pig BW and pen feed disappearance were collected weekly on dpi 0, 7, 14, 21, 28, 35, and 42 to calculate ADG, ADFI and G:F. Pigs were allowed unrestricted access to feed and water throughout the 42 d PRRSV challenge. All pigs were then fed a common diet for eight days prior to beginning Exp. 2.

Experiment 2, MHP Challenge in 80 to 123 kg BW Pigs

Following the 42 d PRRSV challenge (Exp. 1) and eight days of a common diet, a total of 464 mixed sex pigs (79.57. ± 8.97 kg BW) were allotted to one of two dietary treatments with 12 pens (9 to 10 pigs per pen) per dietary treatment per MHP status. The two treatments were 1) control, a diet formulated to contain 1.95 g SID Lys:ME (representing 100% Lys:ME) and 2) a high Lys diet containing 2.34 g SID Lys:ME achieved via the increased inclusion of SBM (120% ratio from control). The two diets were formulated to contain 1.95 and 2.34 g SID Lys:ME, representing 100% and 120% of requirement for 75 to 135 kg BW pigs and validated internally

by the Maschhoffs LLC. The three diets were formulated to contain similar total calcium, available phosphorus and ratios of SID Thr, Trp, Met, Ile, and Val to SID Lys to avoid secondary AA deficiencies (Table 3.3).

On d 106 post-weaning (8 days following the conclusion of Exp. 1) corresponding dpi 0 of MHP inoculation, one barn containing 240 pigs was inoculated with MHP, administered by aerosol fogging of saline diluted MHP infected lung material homogenate. The fogging solution contained 200 mL of MHP infected lung homogenate diluted into 7.6 L of phosphate buffered solution (**PBS**). The second barn with the remaining 224 pigs, was not inoculated with MHP, serving as a control for the test period. For the next 40 dpi pig BW and pen feed disappearance were collected approximately every two weeks at dpi 12, 28 and 40 to calculate ADG, ADFI and G:F. Pigs were allowed unrestricted access to feed and water throughout the 40 d MHP challenge. At the conclusion of Exp. 2, all pigs were marketed to a commercial packing plant (no carcass data was collected).

Diet Analysis

The nine diets fed during the nursery period, three experimental PRRSV and two experimental MHP diets were analyzed for energy and nutrient composition. Proximate analysis of dietary gross energy (**GE**) content was determined using bomb calorimetry (Oxygen Bomb Calorimeter 6200, Parr Instruments, Moline, IL). Diet samples were analyzed for dietary dry matter (**DM**) using method 934.01 according to AOAC (2007). Dietary nitrogen (**N**) analysis of the seven experimental nursery diets were analyzed using a TruMac N (Leco Corporation, St. Joseph, MO). Dietary AA and N analysis of the three experimental PRRSV diets and two experimental MHP diets were conducted by Experimental Station Chemical Laboratories

(Columbia, MO). Amino acid and N analysis were performed using method 994.12, 999.13, and 990.03 according to AOAC (2007) methods, and crude protein (**CP**) was calculated (N × 6.25).

Experiment 1, PRRSV Challenge Blood Collection and Analysis

Two pigs in each pen were randomly selected and these two same pigs were snare-restrained and serial bled on dpi -7, 0, 7, 14, 21, 28, 35 and 42. Blood samples (8-10 mL) were collected from the jugular vein into serum tubes (BD Vacutainer, Franklin Lakes, NJ) for routine diagnostic testing. All blood samples were allowed to clot, then serum separated by centrifugation (2,000 × *g*, 15 min at 4°C), aliquoted and stored at -80˚C until analysis at the ISUVDL. Real-time polymerase chain reaction (**RT-PCR**) and serum antibody testing for PRRSV was performed using commercial reagents (VetMAX™ NA and EU PRRSV RT-PCR, Thermo Fisher Scientific, Waltham, MA) and a commercial ELISA kit (HerdCheck® PRRS X3, IDEXX Laboratories, Inc., Westbrook, ME), respectively. A serum viremia cycle threshold (**Ct**) ≥ 37 was considered negative and serology antibody was considered negative when sample-to-positive (**S:P**) ≤ 0.40.

Experiment 2, MHP Challenge Deep Tracheal Swab Collection and Analysis

Ten pigs in each barn were chosen at random for sample collection. Pigs were snare-restrained for deep tracheal swab collection on dpi -7, 12, 27 and 40 from MHP inoculation. Tracheal swabs were collected using a flocked swab and a modified post cervical artificial insemination (**PCAI**) rod (inner catheter of a PCAI rod) and placed into a 5 mL falcon tube with 3 mL of saline solution then immediately submitted for diagnostic testing. Samples were collected to confirm MHP status of the two barns, thus were pooled within barn and submitted to the ISUVDL, Ames, IA. An MHP viremia Ct ≥ 37 was considered negative.

Statistical Analysis

Pen was considered the experimental unit, with all data analyzed utilizing a complete randomized design using the PROC MIXED procedure in Statistical Analysis System (**SAS**) 9.4 (SAS Inst. Inc., Cary, NC). All performance data from the nursery and Exp. 1 were analyzed for the fixed effects of dietary treatment, vaccination and their interaction, and barn treated as a random effect in the model. Dietary treatments during the nursery phase consisted of low, medium and high SBM inclusion levels. The nursery diets had no effect on the performance if pigs prior to Exp. 1 and 2, and therefore nursery SBM inclusion level was not included in the analysis models for these two experiments. During the PRRSV challenge period of the study (Exp. 1), dietary treatments consisted of control, HL and HF, representing 2.98, 3.57, 3.57 g SID Lys:ME, respectively. Least-squares (**LS**) means of treatment, vaccination and their interaction were determined using the LS means statement, and differences in LS means were produced using the pdiff option. During the MHP challenge period (Exp. 2), data was analyzed individually between barns (MHP status), using fixed effects of dietary treatment (100% and 120% SID Lys:ME of requirement) in the model. Least-squares means of dietary treatment were determined using the LS means statement, and differences in LS means were produced using the pdiff option. Tukey's multiple comparison adjustment was used on all LS mean pairwise comparison. All data was reported as LS means and standard error of the mean (**SEM**). Differences were considered significant when $P < 0.05$ and a tendency when $0.05 < P < 0.10$.

Results

Diet Analysis

Experimental diets were formulated to contain 2.98, 3.57, 3.57 and 1.95 and 2.34 g SID Lys per Mcal ME in Exp. 1 and 2, respectively (Table 3.2 and 3.3, respectively). Proximate analysis of the nursery and experimental diets determined that diets were formulated similar to the predicted values (Table 3.1, 3.2 and 3.3). Analyzed GE of Exp 1. diets were 4.06, 4.07 and 3.92 Mcal/kg, representing the control, HL and HF dietary treatments, respectively. These results confirmed a reduction in dietary energy in the HF diet in comparison to the control and HL diets. As expected, CP increased as SBM inclusion increased.

Vaccination and Nursery Performance

Growth performance data throughout the 42 d nursery period is shown in Table 3.4. Throughout the nursery period vaccine and dietary treatment by vaccine interaction did not have an effect on phase or overall growth performance ($P > 0.05$). During Phase 1, ADG and ADFI was greatest in high SBM fed pigs, with medium SBM pigs being intermediate and low SBM fed pigs having the lowest ADG and ADFI ($P < 0.05$). However, throughout phase 1 G:F feed and end BW did not different between dietary treatment ($P > 0.05$). Throughout phase 2, a tendency was observed for ADG to be increased in low SBM fed pigs compared to medium and high SBM pigs ($P < 0.10$). Low SBM fed pigs had an increased G:F compared to medium and high SBM pigs ($P < 0.05$). End BW and ADFI did not differ throughout phase 2 in response to dietary treatment ($P > 0.05$). Throughout phase 3 (common diet to all pigs), no growth performance parameters differed between previous treatment groups ($P > 0.05$). When analyzing the overall nursery period, an interaction between dietary treatment and vaccination status was observed for ADG ($P < 0.05$). However, the remaining overall growth performance parameters for the 42 d

nursery period did not differ between dietary treatments or vaccination status ($P > 0.05$). Thus, high SBM inclusion rates in phase 1 and 2 did not hinder pig performance.

Experiment 1

No mortalities were recorded due to the PRRSV challenge in this period. The serology responses to the PRRS vaccine and the PRRSV challenge are reported in Table 3.5. Prior to PRRSV inoculation, all pigs were negative for viremia and both the PRRSV vaccinated and non-vaccinated pigs did not have detectable PRRSV antibodies. The success of the viral challenge was assessed via PCR weekly throughout the 42 d challenge period. By 7 dpi, irrespective of dietary treatment and vaccination status, PRRSV viremia Ct values were considered positive and increased with time (i.e. viremia decreased) as pigs seroconverted. Irrespective of vaccination status, PRRSV antibody levels increased throughout the challenge period and plateaued between dpi 25 and 42, at which time all pigs were considered non-viremic (Table 3.5).

During the 13 d acclimation period to grower pens, no differences in ADG or G:F were observed ($P > 0.05$; Table 3.6). However, during this period ADFI was increased in the non-vaccinated pigs in comparison to vaccinated ($P < 0.05$; Table 3.6). Weekly growth performance during the PRRSV challenge period is reported in Table 3.6. From 0 to 7 dpi, ADFI increased in the HF fed pigs compared to the control pigs ($P = 0.05$), while the HL did not differ from either treatment. Also, in this time period non-vaccinated pigs had a tendency for increased ADFI in comparison to vaccinated pigs ($P = 0.067$). On d 0 and 7 non-vaccinated pigs had increased BW compared to vaccinated pigs ($P < 0.01$). Growth rates between dietary treatments were similar from dpi 7 to 14 ($P > 0.10$); however, G:F of vaccinated pigs was increased 0.093 kg above non-vaccinated pigs ($P < 0.05$). In week 3, ADFI increased 10.7% and 17.2% in pigs fed HL and HF diets, respectively in comparison to control ($P < 0.05$). All other performance parameters during

this time period were similar across dietary treatments and vaccination status. During the third week post inoculation, a tendency for G:F and end BW to increase in HL fed pigs in comparison to control with HF differing from neither diet ($P < 0.10$). From dpi 28 to 35 both ADG was greatest in control pigs, intermediate in HL pigs and lowest in HF pigs ($P < 0.05$); with no differences in ADFI seen. However, G:F was greatest in control and HL pigs compared to HF pigs ($P < 0.05$). In the last week of the PRRSV challenge, ADG increased 18.9% and 5.7% in control and HL fed pigs, respectively in comparison to HF pigs ($P < 0.05$). The HF pigs had the greatest ADFI, while HL pigs were intermediate and control having the lowest ADFI ($P < 0.05$). The greatest G:F reported during the sixth week was the control pigs in comparison to HL and HF pigs ($P < 0. 05$). Additionally, a tendency for nonvaccinated pigs to have increased ADFI in comparison to vaccinated pigs was also observed ($P < 0.10$).

For the overall 42 d PRRSV challenge period, vaccination did not have an effect on ADG and ADFI; however, a tendency for vaccinated pigs to have increased G:F compared to non-vaccinated pigs was observed ($P < 0.10$). A tendency was also observed for HL pigs to have the greatest ADG, compared to the control pigs being intermediate and the HF pigs having the lowest ($P < 0.10$). Overall ADFI was increased 8.6 and 3.6% in HF and HL pigs, respectively compared to control ($P < 0.05$). An increase in overall G:F was observed in pigs fed control and HL diet compared to HF, with a 13.3% and 11.2% increase, respectively ($P < 0.05$). Body weights at dpi 42 did not differ between dietary treatment or vaccination status ($P > 0.05$; Table 3.6).

Experiment 2

In Exp. 2, deep tracheal swabs were collected on dpi -7, confirming that both barns were in fact MHP negative (Ct ≥ 37.0). Swabs were collected again on dpi 12 and 27 dpi confirming

that the MHP inoculated barn was positive (Ct values of 28.0 and 24.7, respectively; Ct ≥ 37.0 represents MHP negative), indicating a successful MHP inoculation. The control barn was also was confirmed negative (Ct ≥ 37.0) on dpi 12 and 40, confirming that a negative MHP status was maintained for the entirety of the 40 d challenge period in the control group. Within inoculation status, growth performance response to the 100% and 120% Lys:ME diets throughout the 40 d MHP challenge period is presented in Table 3.7. No differences in ADG, ADFI or G:F were reported due to dietary treatment in the non-challenged pigs ($P > 0.05$; Table 3.7). Within the MHP challenged pigs, increasing the dietary Lys:ME also resulted in no differences in ADG, ADFI or G:F during the 40 d period ($P > 0.05$).

Discussion

Inevitably in modern pig production (i.e. increased pig density and sharing of common airspaces) exposure to potential pathogens will occur. In the event of a viral challenge such as PRRSV, growth rates have been shown to be reduced by 30 to 59%, compared to that of healthy contemporaries (Greiner et al., 2000; Che et al., 2011; Rochell et al., 2015; Schweer et al., 2016). The impact and severity that PRRSV has on growth performance is influenced by factors such as pig age, duration of study, viral strain and viral clearance (Murtaugh et al., 2010). Recently, nutritional strategies have been shown to augment pig growth performance during PRRSV challenges (Rochell et al., 2015; Schweer et al., 2018; Jasper et al., 2020). Herein, we further evaluated formulation approaches to increase SID Lys:ME in PRRSV and MHP challenged pigs to augment growth performance during a health-challenge.

Considering healthy pigs have the ability to alter their feed intake to meet their energy needs (Baker et al., 1968), commonly diets are formulated on an essential AA to energy ratio

(i.e. g SID Lys:ME) ensuring sufficient levels of AA and energy are available for optimal growth. Previous work from our group (Schweer et al., 2018), reported utilizing breakpoint analysis that during both experimental and natural PRRSV challenges in grower pigs, increasing SID Lys:ME 110% to 120% above requirement resulted in improved growth performance and feed efficiency. The benefit of an increased Lys:ME was thought to be attributed to reducing the impact of depressed feed intake (i.e. Lys intake), as reductions in ADFI are typically reported in PRRSV challenged pigs (Greiner et al., 2000; Che et al., 2011; Schweer et al., 2016). The beneficial performance reported when increasing dietary Lys:ME in PRRSV challenged pigs has been further validated by our group (Jasper et al., 2020) utilizing two formulation approaches; either increasing SID Lys via increased inclusion of SBM or by diluting ME via the inclusion of fine grade sand. Improved growth performance in PRRSV challenged pigs was observed irrespective of formulation approach used to achieve 120% Lys:ME (Jasper et al., 2020). However, the use of sand as a feedstuff to dilute dietary energy is not practical from a farm management standpoint. Thus, a more practical feedstuff (i.e. fiber) could be used to dilute energy in commercial swine production.

To evaluate industry feasible formulation strategies to achieve 120% SID Lys:ME (Exp. 1), diets were formulated by either increasing SID Lys (HL) or reducing energy with a fibrous feedstuffs (HF). In comparison to the control diet, the HL diet was achieved via increased inclusion of SBM. Alternatively, the HF diet was achieved via slightly increased inclusion of SBM in addition to the inclusion of soybean hulls. Although SBM contains naturally occurring bioactive components (i.e. isoflavones) that have previously displayed antiviral activity in the face of PRRSV (Greiner et al., 2001; Rochell et al., 2015; Smith et al., 2019), in Exp. 1 no numerical differences in viremia (i.e. PCR Ct values) or PRRSV antibody titers were observed

due to dietary treatment. By design, the increased inclusion of SBM in the various experimental diets is likely increasing multiple essential and non-essential AA in the diet. This increase in AA may be beneficial during a health-challenge (Reeds and Jahoor, 2001; Litvak et al., 2013; Rochell et al., 2015) by reducing the need for lean tissue catabolism, thus preserving lean tissue.

Reductions in feed intake during a disease challenge (Pastorelli et al., 2012) reduce the amount of nutrients available to tissues, thus being the primary cause of reduced lean tissue accretion observed during a viral challenge (Helm et al., 2019). In the event of a PRRSV challenge, feed intake is typically reduced by 25 to 30% (Rochell et al., 2015; Schweer et al., 2016); however, in severe PRRSV challenges this can be upwards of a 40-60% reduction in feed intake (Escobar et al., 2006; Helm et al., 2020). In the current study, feed intake was reduced during the first two weeks post inoculation, as the impact that PRRSV has on feed intake lessen as time progressed (Schweer et al., 2016; Schweer et al., 2017). Metabolizable energy was reduced 8.2% via the inclusion of 8.3% soy hulls in the HF diet, consequently increasing the fiber content. Soy hulls are a by-product of SBM processing and contain around 75% of non-starch polysaccharides, of which 60% are insoluble fiber typically equating to an neutral detergent fiber (**NDF**) of 56% (Kornegay, 1978; Lo, 1990). These non-starch polysaccharides components cannot be broken down efficiently in the small intestine of pigs, thus passing into the large intestine where microbial fermentation then occurs (Velayudhan et al., 2015). In pigs, feeding a high fiber diets normally has negative effects on voluntary feed intake, as high fiber diets decrease the rate of gastric emptying, thus contributing to earlier satiety (Kerr and Shurson, 2013). However, in the current study, similar to results reported by Jasper et al. (2020), overall ADFI increased 8.6% in HF fed PRRSV challenged pigs (Exp. 1). Collectively indicating that pigs fed the HF diet displayed the ability to adjust their voluntary feed intake to consume

additional feed to satisfy their energy needs. Thus, the HF diet could be a possible dietary mitigation strategy to consider when feeding PRRSV challenged pigs to help mitigate disease associated anorexia (i.e. improve feed intake) without suppressing feed intake.

A second objective of this work presented herein (Exp. 2), was to evaluate if increased SID Lys:ME could be a beneficial nutritional strategy in the event of a non-viral health-challenge, such as MHP. Various MHP vaccines are available including inactivated and live attenuated vaccines that can be administered to help mitigate negative performance associated with a MHP challenge; however varying vaccines efficacies have been reported (Tao et al., 2019). Commonly, MHP vaccinations are administer prior to weaning, thus by late finishing vaccine coverage fluctuates in which understanding possible nutritional mitigation strategies to attenuate disease is crucial. However, the specific AA requirements of pigs undergoing an MHP challenge are widely undetermined, thus it was unknown if a 120% Lys:ME would be advantageous in augmenting growth in late finishing pigs.

In the current study, during an experimental late finishing MHP challenge SID Lys was increased via increased inclusion of SBM, to achieve a 120% Lys:ME. However, unlike previously reported results in PRRSV challenged pigs, no differences in growth performance parameters or end BW were observed in comparison to control 100% Lys:ME fed pigs undergoing an MHP challenge. These data suggest that MHP vaccinated pigs undergoing a mild experimental MHP challenge (via aerosol fogging) may not benefit similarly to increased Lys:ME as reported in PRRSV vaccinated and non-vaccinated pigs undergoing a PRRSV challenge. Nevertheless, Surendran Nair et al. (2019) has shown that non-proteogenic AA (often considered the catabolic product of other amino acids) have been found at significantly increased levels during a MHP challenge, indicating a shift in AA metabolism occurring. Additionally, the

lack of difference in growth performance could possibly be accounted for by reduced AA needs for lean tissue growth in late finishing. In growing pigs protein deposition (**PD**) increases rapidly at low body weights, while at higher body weights (i.e. grow-finish stage) PD tends to plateau (de Lange et al., 2001). The upper limit of daily protein deposition (PD_{MAX}) is largely influenced by genotype, sex and body weight (NRC, 2012). Thus, indicating that an increased Lys:ME ratio in heavier late finishing may not be as beneficial as previously seen in early grow-finish pigs, as PD_{MAX} may have been reached prior to experimental diet implementation. Therefore, Lys, Met, Thr, Trp and other AA may not be beneficial at increased levels in the diet in late finishing.

In comparison to previous PRRSV challenge studies our group has conducted, Exp. 1 utilized an intranasal inoculation route to more closely mimic a natural challenge. Consequently, clinical signs typically observed throughout a PRRSV challenge were delayed. Additionally more moderate growth reductions were observed in the current study in comparison to previous intramuscular PRRSV inoculation studies from our group (Schweer et al., 2018; Jasper et al., 2020). Similar results have been reported by Yoon et al. (1996) when comparing intramuscular and intranasal inoculation routes, with a more uniform viral response observed in intramuscular inoculated pigs. Significant reductions in ADG and ADFI have previously been reported by our group when utilizing intramuscular inoculation with the same viral strain of PRRSV as used in the current study and a comparable size pig (Schweer et al., 2017; Schweer et al., 2018; Jasper et al., 2020). However, the overall reductions in ADG and ADFI in Exp. 1 and 2 were not as significantly reduced when utilizing an intranasal inoculation method. In the case of PRRSV, the virus typically infiltrates the respiratory tract and resides in the porcine alveolar macrophages (Duan et al., 1997; Zhang and Yoo, 2015). When utilizing intranasal inoculation, PRRSV must

penetrate and cross the primary line of defense consisting of mucosal barriers in the respiratory tract, potentially increasing inoculation variability.

In today's swine production, commonly herds are vaccinated against PRRSV to help mitigate the negative effects associated with a PRRSV infection, with both modified live vaccines (**MLV**) and autogenous vaccines from field isolates in use. However, research trials and field studies with these commercially available vaccines have found considerable variation in efficacy (Osorio et al., 1998 ; Mavromatis et al., 1999; Oh et al., 2019). In addition, mucosal vaccines targeting the immune cells located near the mucosal surfaces were the initial viral infection often occurs are also becoming readily available; however, limited data of their efficacy is available. Results from Exp. 1 suggest that the killed mucosal PRRSV vaccine used did not improve growth performance during a PRRSV challenge, as no differences in growth performance between vaccination statuses was observed. Thus, further research is warranted in regard to mucosal PRRSV vaccinations and the potential effect it may have on modulating mucosal and systemic immunity to mitigate negative growth performance during a PRRSV challenge.

In summary, this work supports that during an experimental PRRSV challenge, increasing SID Lys:ME to 120% in grower pigs aids in the mitigation of negative growth performance throughout the challenge period (Schweer et al., 2018; Jasper et al., 2020). However, in the current study due to a mild PRRSV response (i.e. moderate growth reduction), overall ADG and end BW did not differ in response to dietary treatment or vaccination status. A dilution of energy in the diet (HF) resulted in increased feed intake yet did not translate to an increase in ADG in comparison to control, as the PRRSV challenge was moderate. In the event of an MHP challenge, feeding an 120% Lys:ME diet had no effect on growth performance or end

BW in late finishing MHP vaccinated pigs. Further work is needed to identify dietary mitigation strategies in MHP challenged pigs, as MHP is a highly variable bacterial challenge. Nevertheless, the results from this study support the notion that in the event of a stressor such as a disease challenge AA requirement alter, which is likely due to increased metabolic activity and repartitioning of nutrients away from lean tissue accretion. Indicating the importance of determining nutrient requirements of health challenged pigs.

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Table 3.1. Nursery diet composition of low, medium, and high soybean meal, as fed basis

Ingredient %	Phase 1			Phase 2			Phase 3
	Low	Medium	High	Low	Medium	High	Common
Corn, yellow dent	44.91	39.58	33.63	58.84	49.34	39.64	53.08
Soybean meal (46% CP)	15.00	25.00	35.00	25.00	35.00	45.00	27.60
Whey Powder	24.25	24.25	24.25	-	-	-	-
HP300	8.53	4.19	1.55	-	-	-	-
DDGS	-	-	-	10.00	10.00	10.00	15.00
Monocalcium phosphate (21%)	1.48	1.49	1.47	1.78	1.71	1.65	0.50
Limestone	1.00	0.95	0.90	1.11	1.10	1.14	1.43
Salt	0.78	0.77	0.75	0.76	0.75	0.73	0.50
Spray Dried Plasma	0.75	0.75	0.75	-	-	-	-
Wheat Gluten	1.00	1.00	-	-	-	-	-
Soybean Oil	0.50	0.50	0.50	0.55	0.84	1.10	-
Fat Animal-Vegetable Blend	-	-	-	-	-	-	0.50
Zinc oxide, 72% Zn	0.39	0.39	0.39	0.25	0.25	0.25	0.11
Dairy Protein, 55%	0.25	0.25	0.25	0.10	0.10	0.10	-
Copper Sulfate	0.07	0.07	0.07	0.07	0.07	0.07	-
L-Lysine Sulfate (54.6%)	0.40	0.25	0.05	0.61	0.30	-	-
DL-Methionine	0.20	0.16	0.12	0.20	0.11	0.03	-
L-Threonine	0.16	0.10	0.03	0.26	0.12	-	-
L-Tryptophan	0.02	-	-	0.06	-	-	-
L-Isoleucine	-	-	-	0.02			-
L-Valine	-	-	-	0.09	-	-	-
Trace mineral premix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.10
Vitamin premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.04
Other[3]	-	-	-	-	-	-	0.24
Calculated composition							
DM, %	87.55	87.44	87.36	87.59	87.73	87.89	87.22
CP, %	20.21	21.79	23.45	21.16	23.51	26.97	21.41
ME, Mcal/kg	3.27	3.25	3.22	3.16	3.16	3.16	3.47
Total Calcium, %	0.90	0.90	0.90	0.85	0.87	0.90	0.77
Total, Phosphorus, %	0.77	0.80	0.83	0.80	0.83	0.86	-
DIG AA							
Lys	1.30	1.30	1.30	1.30	1.30	1.30	1.20
Thr:Lys	0.65	0.65	0.65	0.65	0.65	0.66	0.60
Met+Cys:Lys	0.58	0.58	0.58	0.58	0.58	0.58	0.57
Trp:Lys	0.20	0.20	0.23	0.19	0.19	0.23	0.18
Ile:Lys	0.60	0.65	0.72	0.54	0.65	0.78	0.59
Val:Lys	0.65	0.70	0.76	0.65	0.71	0.83	0.65
SID Lys:ME, g/Mcal	3.98	4.01	4.04	4.11	4.11	4.12	-
Lys, Total %	1.42	1.43	1.45	1.30	1.30	1.30	1.36
Analyzed composition							
DM, %	91.52	91.37	91.11	90.50	89.85	90.10	90.34
CP, %	19.07	21.35	22.12	19.29	22.39	26.70	21.54
GE, Mcal/kg	3.79	3.78	3.77	3.81	3.85	3.89	3.97

[1]Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 4,593.75 IU; vitamin D3, 525; vitamin E, 37.5 IU; vitamin K 2.25 mg; niacin, 42 mg; pantothenic acid, 20.25 mg; riboflavin, 8.25 mg; Vitamin B12, 0.0375 mg

[2]Provided the following quantities of trace minerals per kilogram of complete diet: Cu, 9 mg as copper sulfate; I, 0.2 mg as calcium iodate; Fe, 120 mg as copper sulfate; Mn, 6.75 mg as manganese sulfate; Se, 0.23 mg as sodium thiosulfate; Zn, 120 mg as zinc sulfate.

[3]Other: Phytase 500FTU/KG, Skysis 100, Copper Chloride, Engage M, Hemicell HT

Table 3.2. Experiment 1 PRRSV diet composition, as fed basis, 33 to 70 kg BW pig

Ingredients, %	g SID[1] Lys:Mcal ME		
	2.98 (control)	**3.57 (HL)**	**3.57 (HF)**
Corn	58.22	50.00	50.07
Soybean meal (48% CP)	20.51	29.09	23.89
DDGS	15.00	15.00	15.00
Soy hulls	-	-	8.34
Limestone	1.19	1.19	1.07
Monocalcium phosphate (21%)	0.35	0.16	0.29
Salt	0.40	0.40	0.41
Fat, Animal-Vegetable Blend	3.41	3.19	0.00
L-Lysine Sulfate (54.6%)	0.58	0.58	0.58
L-Threonine	0.06	0.07	0.07
DL-Methionine	0.08	0.13	0.11
Engage M	0.05	0.05	0.05
Vitamin Premix[2]	0.03	0.03	0.03
Trace Mineral Premix[3]	0.08	0.08	0.08
Copper Chloride (25.2%)	0.03	0.03	0.03
Phytase 500FTU/KG	0.01	0.02	0.01
Calculated composition			
DM, %	87.25	87.41	87.18
CP, %	18.30	21.68	20.27
ME, Mcal/kg	3.31	3.31	3.04
Acid Detergent Fiber, %	4.17	4.43	7.56
Neutral Detergent Fiber, %	9.80	9.86	14.04
Total Calcium, %	0.63	0.63	0.63
P, Available %	0.31	0.31	0.31
Lys, Total %	1.13	1.35	1.26
SID AA			
Lys	0.99	1.18	1.09
Thr:Lys	0.60	0.60	0.60
Met+Cys:Lys	0.57	0.57	0.57
Trp:Lys	0.17	0.19	0.17
Ile:Lys	0.60	0.62	0.61
Val:Lys	0.68	0.68	0.67
SID Lys:ME, g/Mcal	2.98	3.57	3.57
Analyzed composition			
DM, %	89.65	89.77	89.61
CP, %	18.99	21.69	21.01
GE, Mcal/kg	4.06	4.07	3.92
Lys, Total %	1.03	1.00	1.06
Total AA:Lys			
Thr:Lys	0..67	0.71	0.70
Met+Cys:Lys	0.71	0.67	0.72
Ile:Lys	0.74	0.77	0.80
Val:Lys	0.86	0.87	0.93

[1]SID = standard ileal digestibility

[2]Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 5,291 IU as vitamin A acetate; vitamin D3, 827 IU as vitamin D-activated animal sterol; vitamin E, 26 IU as α-tocopherol acetate; menadione, 1.5 mg as menadione dimethylpyrimidinol bisulfite; vitamin B12, 0.02 mg; riboflavin, 6.0 mg; pantothenic acid, 22 mg as calcium pantothenate; niacin, 30 mg.

[3]Provided the following quantities of trace minerals per kilogram of complete diet: Fe, 124 mg as iron sulfate; Zn, 124 mg as zinc oxide; Mn, 29 mg as manganese sulfate; Cu, 12 mg as copper sulfate; I, 0.22 mg as calcium iodate; and Se, 0.22 mg as sodium selenite.

Table 3.3. Experiment 2 *Mycoplasma hyopneumoniae* (MHP) diet composition, as fed basis, 80 to 123 kg BW pig

Ingredients, %	g SID[1] Lys:Mcal ME	
	1.95 (control)	2.34 (HL)
Corn	74.93	69.80
DDGS	15.00	15.00
Soybean meal	7.07	12.27
Limestone	1.12	1.10
Salt	0.44	0.34
Fat, Animal-Vegetable Blend	0.75	0.75
L-Lysine Sulfate (54.6%)	0.49	0.50
Threonine	0.05	0.07
Tryptophan	0.02	0.01
Methionine-DL	-	0.02
Vitamin Premix[2]	0.03	0.03
Trace Mineral Premix[3]	0.08	0.08
Copper chloride (58%)	0.03	0.03
Phytase 500FTU/KG	0.01	0.01
Calculated composition		
DM, %	86.64	86.74
CP, %	13.24	15.31
ME, Mcal/kg	3.83	3.83
Total Calcium, %	0.48	0.49
STTD Phosphorus, %	0.21	0.23
Lys, Total %	0.76	0.90
SID AA		
Lys	0.64	0.77
Thr:Lys	0.64	0.64
Met+Cys:Lys	0.59	0.57
Trp:Lys	0.18	0.18
Ile:Lys	0.60	0.61
Val:Lys	0.73	0.71
SID Lys:ME, g/Mcal	1.95	2.34
Analyzed composition		
DM, %	87.29	87.32
CP, %	14.22	14.73
GE, Mcal/kg	4.00	3.89
Lys, Total %	0.72	0.97
Total AA:Lys		
Thr:Lys	0.68	0.56
Met+Cys:Lys	0.64	0.52
Ile:Lys	0.72	0.61
Val:Lys	0.82	0.73

[1]SID = standard ileal digestibility

[2]Provided the following quantities of vitamins per kilogram of complete diet: vitamin A, 5,291 IU as vitamin A acetate; vitamin D3, 827 IU as vitamin D-activated animal sterol; vitamin E, 26 IU as α-tocopherol acetate; menadione, 1.5 mg as menadione dimethylpyrimidinol bisulfite; vitamin B12, 0.02 mg; riboflavin, 6.0 mg; pantothenic acid, 22 mg as calcium pantothenate; niacin, 30 mg.

[3]Provided the following quantities of trace minerals per kilogram of complete diet: Fe, 124 mg as iron sulfate; Zn, 124 mg as zinc oxide; Mn, 29 mg as manganese sulfate; Cu, 12 mg as copper sulfate; I, 0.22 mg as calcium iodate; and Se, 0.22 mg as sodium selenite.

Table 3.4. Overall effects of increased soybean meal on BW and growth performance in nursery pigs

Parameter[6]	SBM Diets			SEM	Vaccine		SEM	*P*-values		
	Low	Medium	High		+	-		SBM	VAC	SBM x VAC
Start BW, kg	5.52	5.53	5.67	0.128	5.59	5.56	0.104	0.650	0.879	0.954
Phase 1[2]										
ADG, kg	0.201[b]	0.219[ab]	0.229[a]	0.006	0.209	0.223	0.005	0.011	0.068	0.142
ADFI, kg	0.224	0.240	0.249	0.007	0.232	0.243	0.005	0.049	0.198	0.109
G:F	0.900	0.913	0.920	0.018	0.904	0.918	0.014	0.715	0.495	0.899
End BW, kg	7.92	8.16	8.42	0.153	8.10	8.23	0.125	0.096	0.461	0.681
Phase 2[3]										
ADG, kg	0.403	0.375	0.366	0.012	0.380	0.382	0.010	0.094	0.899	0.496
ADFI, kg	0.538	0.541	0.528	0.017	0.527	0.544	0.013	0.840	0.399	0.807
G:F	0.750[a]	0.695[b]	0.693[b]	0.011	0.722	0.703	0.009	0.002	0.138	0.312
End BW, kg	12.39	12.30	12.41	0.240	12.28	12.46	0.196	0.940	0.524	0.530
Phase 3[4]										
ADG, kg	0.564	0.605	0.587	0.014	0.578	0.593	0.011	0.131	0.335	0.452
ADFI, kg	0.888	0.939	0.901	0.018	0.897	0.923	0.015	0.149	0.228	0.841
G:F	0.636	0.645	0.651	0.012	0.644	0.644	0.010	0.681	0.957	0.567
End BW, kg	23.66	24.41	24.11	0.389	23.81	24.31	0.317	0.406	0.275	0.322
Overall[5]										
ADG, kg	0.379	0.400	0.394	0.007	0.389	0.393	0.006	0.142	0.676	0.030
ADFI, kg	0.550	0.573	0.560	0.011	0.552	0.570	0.009	0.327	0.172	0.643
G:F	0.762	0.751	0.754	0.006	0.757	0.755	0.005	0.473	0.774	0.425

[1] PRRS Aptimmune Barricade vaccine administered d 1 and 23
[2] Fed d 0 to 11; treatment low, medium and high representing 15%, 25% and 35% SBM respectively
[3] Fed d 12 to 22; treatment low, medium and high representing 25%, 35% and 45% SBM respectively
[4] Fed d 23 to 42; common nursery diet fed to all treatment groups
[5] Overall nursery performance d 0 to 42
[6]Within dependent variable, means without a common superscript (a-c) differ significantly ($P < 0.05$)

Table 3.5. Pooled viremia and antibody titers in PRRSV challenged pigs fed increased ratio of standardized ileal digestible (SID) lysine to metabolizable energy (ME), Exp. 1

Parameter[2]	g SID[1] Lys:Mcal ME					
	Vaccinated			Nonvaccinated		
	2.98 (control)	3.57 (HL)	3.57 (HF)	2.98 (control)	3.57 (HL)	3.57 (HF)
PRRSV Ct value[3]						
dpi 0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0
dpi 7	23.8	28.9	32.1	31.0	27.7	34.3
dpi 14	25.8	24.4	23.2	23.8	23.3	22.8
dpi 21	29.4	31.1	29.2	30.6	26.9	30.2
dpi 28	33.6	35.3	34.5	32.9	36.6	32.5
dpi 35	≥37.0	≥37.0	36.6	≥37.0	≥37.0	≥37.0
dpi 42	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0	≥37.0
PRRSV S/P ratio[4]						
dpi 0	0.013	0.013	0.158	0.032	0.010	0.039
dpi 7	0.008	0006	0.071	0.005	0.004	0.008
dpi 14	0.712	0.503	0.285	0.784	1.102	0.423
dpi 21	1.758	1.692	1.617	1.553	1.712	1.690
dpi 28	1.834	1.774	1.599	1.746	1.695	1.681
dpi 35	1.820	1.754	1.774	1.703	1.752	1.611
dpi 42	1.694	1.823	1.668	1.698	1.788	1.695

[1]SID = standardized ileal digestibility
[2]Pooled serology within treatment and vaccine status
[3]Cycle threshold (Ct), Ct ≥ 37.0 denotes PRRS negative.
[4]PRRSX3 antibody sample to positive (S/P) ratio, ≤ 0.40 denotes PRRS negative.

Table 3.6. Effects of increasing the ratio of standardized ileal digestible (SID) lysine to metabolizable energy (ME) on growth performance in PRRSV challenged growing pigs, Exp. 1

Parameter[7]	g SID[1] Lys:Mcal ME[2]			SEM	Vaccine[3]		SEM	*P*-values		
	2.98 (control)	3.57 (HL)	3.57 (HF)		+	-		TRT	VAC	TRT x VAC
Transition[4]										
Start BW, kg	23.7	24.4	24.1	0.389	23.8	24.3	0.317	0.406	0.275	0.322
ADG, kg	1.416	1.420	1.480	0.037	1.412	1.465	0.032	0.429	0.231	0.480
ADFI, kg	2.589	2.685	2.683	0.179	2.579^{y}	2.726^{x}	0.177	0.319	0.018	0.630
G:F	0.545	0.526	0.548	0.065	0.545	0.535	0.064	0.675	0.672	0.445
PRRSV Challenge[5]										
dpi 0 to 7										
Start BW, kg	33.7	34.4	34.4	0.360	33.6^{y}	34.7^{x}	0.29	0.301	0.012	0.574
ADG, kg	0.952	0.965	0.930	0.068	0.916	0.982	0.064	0.836	0.187	0.949
ADFI, kg	1.646^{b}	1.712^{ab}	1.787^{a}	0.053	1.673	1.758	0.048	0.050	0.067	0.785
G:F	0.577	0.565	0.518	0.029	0.545	0.561	0.026	0.150	0.539	0.807
End BW, kg	40.1	41.1	40.9	0.710	40.0^{y}	41.4^{x}	0.656	0.288	0.014	0.394
dpi 7 to 14										
ADG, kg	0.558	0.631	0.549	0.042	0.610	0.549	0.036	0.309	0.210	0.181
ADFI, kg	1.296	1.394	1.377	0.070	1.309	1.402	0.058	0.621	0.277	0.874
G:F	0.431	0.461	0.399	0.024	0.465^{x}	0.396^{y}	0.020	0.208	0.023	0.220
End BW, kg	44.0	45.6	44.8	0.903	44.4	45.1	0.837	0.194	0.290	0.798
dpi 14 to 21										
ADG, kg	0.718	0.723	0.720	0.030	0.710	0.730	0.025	0.991	0.564	0.786
ADFI, kg	1.433^{b}	1.587^{a}	1.680^{a}	0.042	1.575	1.558	0.035	0.001	0.725	0.655
G:F	0.505	0.459	0.441	0.021	0.458	0.479	0.017	0.100	0.380	0.779
End BW, kg	48.9	50.6	49.9	0.959	49.4	50.2	0.902	0.118	0.192	0.647
dpi 21 to 28										
ADG, kg	0.716	0.796	0.792	0.033	0.775	0.762	0.027	0.153	0.730	0.348
ADFI, kg	1.897	1.867	1.999	0.056	1.912	1.930	0.046	0.207	0.771	0.542
G:F	0.377^{b}	0.428^{a}	0.398^{b}	0.019	0.404	0.398	0.016	0.049	0.727	0.604
End BW, kg	54.0	56.1	55.6	0.880	54.8	55.7	0.804	0.053	0.241	0.482

Table 3.6. continued

Parameter[7]	g SID[1] Lys:Mcal ME[2]			SEM	Vaccine[3]		SEM	*P*-values		
	2.98 (control)	3.57 (HL)	3.57 (HF)		+	-		TRT	VAC	TRT x VAC
dpi 28 to 35										
ADG, kg	1.200[a]	1.157[ab]	0.998[b]	0.048	1.147	1.090	0.039	0.011	0.300	0.121
ADFI, kg	2.256	2.281	2.414	0.058	2.328	2.306	0.046	0.118	0.745	0.917
G:F	0.535[a]	0.508[a]	0.412[b]	0.018	0.496	0.475	0.015	<0.001	0.311	0.122
End BW, kg	62.4	64.3	63.0	1.033	62.9	63.6	0.951	0.177	0.409	0.912
dpi 35 to 42										
ADG, kg	1.089[a]	0.968[ab]	0.916[b]	0.038	0.962	1.019	0.032	0.009	0.208	0.155
ADFI, kg	2.446[b]	2.496[ab]	2.645[a]	0.066	2.475	2.583	0.061	0.019	0.067	0.425
G:F	0.447[a]	0.389[b]	0.347[b]	0.015	0.392	0.397	0.012	0.001	0.781	0.513
End BW, kg	69.8	71.2	69.5	1.214	69.6	70.7	1.121	0.285	0.238	0.888
Overall[6]										
ADG, kg	0.856	0.878	0.830	0.024	0.856	0.853	0.022	0.090	0.903	0.847
ADFI, kg	1.826[b]	1.892[ab]	1.983[a]	0.039	1.884	1.917	0.035	0.002	0.337	0.879
G:F	0.475[a]	0.466[a]	0.419[b]	0.007	0.459	0.448	0.007	<0.001	0.069	0.768

[1]SID = standardized ileal digestibility

[2]n = 16 pens/dietary treatment and 9 to 10 pigs/pen

[3]n = 24 pens/ vaccination status; vaccinated d 1 post-weaning and a second time 24 d post-weaning with Aptimmune Barricade mucosal vaccine

[4]Transtion period between nursery and PRRSV challenge period (-13 to -1 dpi); all pigs fed common diet

[5]PRRSV challenge period (0 to 42 dpi), all pigs fed experimental diets

[6]Overall challenge period (0 to 42 dpi), all pigs fed experimental diets

[7]Within dependent variable, means without a common superscript (a-c or x-y) differ significantly ($P < 0.05$)

Table 3.7. Effects of increasing the ratio of standardized ileal digestible (SID) lysine to metabolizable energy (ME) on growth performance in *Mycoplasma hyopneumoniae* (MHP) challenged pigs, Exp. 2

Parameter	g SID[1] Lys:Mcal ME[2]		SEM	*P*-value
	1.95 (control)	2.34 (HL)		
Control[3]				
Start BW, kg	80.2	79.6	0.95	0.67
End BW, kg	125.1	123.6	1.12	0.36
ADG, kg	1.12	1.17	0.054	0.515
ADFI, kg	3.31	3.30	0.043	0.862
G:F	0.34	0.35	0.016	0.460
M. Hyopneumoniae[3]				
Start BW, kg	78.9	79.6	0.84	0.57
End BW, kg	122.6	122.5	0.98	0.94
ADG, kg	1.08	1.07	0.020	0.594
ADFI, kg	3.31	3.32	0.048	0.835
G:F	0.33	0.32	0.006	0.493

[1]SID = standardized ileal digestibility

[2]n = 12 pens/ dietary treatment with 8-10 pigs/pen

[3]Overall MHP challenge period (0 to 40 dpi), pigs fed experimental diets

CHAPTER 4. GENERAL CONCLUSION

Viral and bacterial pathogens impact pig survivability and performance in all stages of swine production worldwide. In the U.S., two commonly reported respiratory pathogens that antagonize grow-finish performance are Porcine reproductive and respiratory syndrome virus (**PRRSV**) and *Mycoplasma hyopneumoniae* (**MHP**). More than 50% of U.S. grow-finish sites are reported positive for PRRSV antibodies and over 55% of U.S. grow-finish sites have reported MHP incidence (NAHMS, 2012). Porcine reproductive and respiratory syndrome (**PRRS**) in the U.S. alone is estimated to cost swine producers upwards of $644 million per year, as it antagonizes all stages of production causing increased morbidity, mortality and reduced growth in grow-finish pigs (Lunney et al., 2010; Holtkamp et al., 2013; Nathues et al., 2017). However, even with endemic diseases such as PRRS and MHP, feeding and managing these challenged pig flows (populations) as well as knowing their nutritional requirements for health recovery and growth performance have remained elusive. Nutritional requirements for healthy pigs are well established by the National Research Council (NRC, 2012); however, nutrient requirements for pigs undergoing a health-challenge are widely unknown, including amino acids (**AA**) requirements. In a healthy pig, Lys is the first limiting AA when feeding corn-soybean meal-based diets. However, the AA utilization of swine with an activated immune system is not as well understood (NRC, 2012).

One nutritional strategy that has been studied to promote earlier viral clearance and recovery, in addition to enhancing pig performance in health challenged pigs is the increased inclusion of soybean meal (**SBM**) (Boyd, 2014). Soybean meal is the main protein and essential AA source in corn SBM-based diets. Rochell et al. (2015) reported that in nursery pigs challenged with PRRSV, increasing SBM from 17.5% to 29% reduced viremia load and

improved growth performance over a 14 d challenge period. However, it is unclear if the improved performance is due to increased dietary crude protein (**CP**) and AA, or the increase in bioactive antioxidant compounds (i.e. isoflavones) found within SBM. The latter has yielded mixed results in modulating PRRSV in challenged pigs (Greiner et al., 2000; Smith et al., 2019). Furthermore, based on previous work from our group, the potential benefits of feeding increased SBM during a PRRSV challenge is likely not related to digestibility of nutrients or AA (Schweer et al., 2018b). This work also highlighted that basal endogenous losses of AA were only nominally different in PRRSV challenged pigs compared to healthy control pigs and this translated to minimal differences in standardized ileal digestibility (**SID**) of most AA (Schweer et al., 2018b).

To further examine the impact of SBM, the impact of the relationship of Lys to energy in PRRSV challenged pigs was evaluated. Utilizing break point analysis Schweer et al. (2018a) reported that increasing dietary Lys:ME to 110% to 120% of requirement improved growth and feed efficiency in PRRSV challenged pigs. The increase in Lys:ME was achieved in the diet primarily with the use of intact protein sources, with synthetic AA levels remaining relatively constant across diets. The relationship of Lys to energy was evaluated because when formulating diets, AA requirements are expressed in relation to energy as a ratio (i.e. SID Lys:ME). This ensures that a constant AA intake is achieved by the pig independent of the dietary energy level fed and related adjustment to feed intake, which is key in supporting optimal feed intake and growth. However, stimulation of the immune system due to a pathogen challenge can result in reduced voluntary feed intake and as a result, lower energy and AA intake (Johnson, 2002; Doeschl-Wilson et al., 2009) causing growth rate suppression (Greiner et al., 2001; Rochell et al., 2015; Schweer et al., 2018a). In addition, it has been suggested that under unrestricted

feeding conditions, healthy pigs will attempt to consume the amount of feed required to satisfy their requirement for energy and nutrients (Schiavon et al., 2018). However, it is unclear if pigs are able to adjust their feed intake to meet their energy needs under stressors such as disease. Therefore, the overall objective of this thesis was to evaluate the importance of increasing dietary SID Lys:ME ratio above requirement (i.e. targeting 120% of requirement) in pathogen challenged pigs to improve growth performance. Further, we also evaluated the formulation approaches used to achieve this increased ratio. To address the overarching objective, three research experiments were conducted and presented in Chapters 2 and 3.

In the first research chapter (Chapter 2), our objective was to evaluate the effects of increasing SID Lys:ME on growth performance in PRRSV vaccinated and nonvaccinated pigs facing a subsequent PRRSV challenge. Furthermore, we hypothesized that irrespective of how the increased Lys:ME ratio (i.e. 120%) was achieved, either by an increase in g SID Lys or a reduction in ME, there would be increased growth performance in PRRSV infected pigs compared to pigs fed a 100% Lys:ME ratio (i.e. at optimal requirement for healthy pigs). Additionally, the reduction in feed intake during a disease challenge reduces nutrient availability to tissues, particularly muscle (Helm et al., 2019), thus being the primary cause of reduced lean tissue accretion observed during a viral challenge (Schweer et al., 2017). Therefore, we also hypothesized that decreasing dietary energy concentration may be beneficial in pigs with an activated immune system resulting in improving feed intake, highlighting the pig's ability to eat to meet their energy needs.

In the first chapter, 393 pigs (35 kg BW) housed with 7 to 10 pigs/pen in two separate barns with one barn being PRRSV vaccinated (**vac+**) while the other remain PRRSV non-vaccinated (**vac-**). On days post inoculation (**dpi**) 0, all pens were randomly allotted onto one of

three dietary treatments with 8 pens per treatment per vaccine status. The three dietary treatments were: 1) control, a diet formulated to contain 2.69 g SID Lys:ME (control diet representing 100% Lys:ME based on NRC 2012); 2) high Lys (**HL**), a diet containing 3.23 g SID Lys:ME achieved via increased inclusion of SBM and synthetic AA (120% ratio from control); and 3) low energy (**LE**), a diet containing 3.22 g SID Lys:ME achieved by reducing dietary ME via the inclusion of 18% fine grade, washed and dried sand (120% ratio from control). Also, on dpi 0 all pigs in both barns were inoculated intramuscularly with 1 mL of live field strain of PRRSV (1-18-4), containing ~10^6 genomic PRRSV units per mL. All pigs tested positive for PRRSV viremia on dpi 7, confirming a successful PRRSV inoculation. Overall, in both PRRSV vac+ and vac- pigs, increasing SID Lys:ME to 120% of requirement during the 42 d PRRSV challenge period increased average daily gain (**ADG**), regardless of how the 120% ratio was achieved, by increasing g SID Lys (HL) or decreasing ME (LE). Overall average daily feed intake (**ADFI**) in LE fed pigs increased 17% and 20% in comparison to control in vac+ and vac- pigs, respectively. In vac+ pigs, dietary treatment had no effect on overall gain-to-feed (**G:F**), however in vac- pigs an increase in overall G:F was observed in pigs fed the HL treatment compared to pigs fed the control and LE treatments, which were not different from each other. In vac+ pigs, end BW of pigs fed HL and LE treatments were improved 5.4 and 5.2 kg, respectively, in comparison to control. Additionally, in vac- pigs, end BW increased in pigs fed HL and LE treatments 6.9 kg and 4.2 kg, respectively, in comparison to control. This study validates Schweer et al. (2018a) studies and proved that during an experimental PRRSV challenge (in addition to a concurrent associated PCV2 challenge), increasing the dietary SID Lys:ME to 120% in grower pigs aids in augmenting growth performance. Additionally, irrespective of vaccination status, diluting ME by 20% with inert sand to achieve a 120% Lys:ME in the diet resulted in increased feed intake,

translating to an increase in ADG and end BW in comparison to control throughout a PRRSV challenge.

The use of sand in diet formulations to dilute dietary energy is not a practical approach or feedstuff from a farm management standpoint. Although sand is an inert in terms of ME, if the sand is too fine, the small micron size could possibly cause irritation to the pig's digestive tract and may settle to the bottom of the pit causing management issues in confinement facilities. Thus, to further evaluate diet formulation strategies to achieve a 120% Lys:ME ratio, PRRSV challenged pigs were fed a diet with reduced dietary energy via dietary fiber source (Chapter 3). It is unknown if altering the Lys:ME by reducing ME utilizing dietary fiber improves performance of PRRSV challenged pigs similarly to the results previously seen in Chapter 2. Therefore, the objective of this study was to further evaluate the formulation approach utilized to achieve a 120% Lys:ME ratio, either by an increase in SID Lys or a reduction in dietary energy via an industry applicable feedstuff in PRRSV challenged grower pigs.

In the second experiment, 464 pigs (~34 kg BW) housed with 9 to 10 pigs/pen in two separate barns. Each barn contained both PRRSV vaccinated (vac+) and PRRSV non-vaccinated (vac-) pigs (12 pens/vaccination status/barn). On dpi 0, all pigs in both barns were randomly allotted to one of three dietary treatments and inoculated intranasally with a virulent, live field strain of PRRSV (1-18-4), administer with a single intranasal 2 mL dose of saline diluted serum containing 10^6 genomic PRRSV units per mL live virulent PRRSV. The three dietary treatments per vaccination status were: 1) control, a diet formulated to contain 2.98 g SID Lys:ME (representing 100% Lys:ME requirement); 2) high Lys (HL), a diet containing 3.57 g SID Lys:ME achieved via increased inclusion of SBM (120% ratio from control) and 3) high fiber (HF), a diet containing 3.57 g SID Lys:ME achieved by reducing dietary ME 8% via the

inclusion of 8.3% soy hulls and increasing Lys 112% via SBM (120% ratio from control). Serum samples representative of all pens had detectable levels of PRRSV in submitted samples via PCR testing at dpi 7, confirming a successful PRRSV inoculation.

Overall, during the 42 d PRRSV challenge period, a tendency was observed for HL pigs to have the greatest ADG (0.878 kg/d), control pigs to be intermediate (0.856 kg/d) and HF pigs the lowest ADG (0.830 kg/d). Overall ADFI was increased 8.6 and 3.6% in HF and HL pigs respectively compared to control, indicating that the HF fed pigs were able to adjust their voluntary feed intake to achieve a higher ADFI than control pigs, in an effort to reach their energy needs; similarly to the result in Chapter 2. Additionally, an increase in overall G:F was observed in pigs fed control and HL diet compared to HF, 13.3 and 11.2% increase respectively. However, end BW at the conclusion of the 42 PRRSV challenge period did not differ between dietary treatments. In summary, experiment 2 supports that during an experimental PRRSV challenge, increasing SID Lys:ME to 120% in grower pigs aids in the mitigation of negative growth performance throughout the challenge period (Schweer et al., 2018a). However, in experiment 2 a relatively mild clinical impact of PRRSV was observed (i.e. a moderate growth performance reduction). Consequently, overall ADG and end BW did not differ between dietary treatments.

In Chapter 2, we concluded that pigs fed increased SID Lys:ME during a PRRS challenge augmented growth performance, in agreeance with Schweer et al. (2018a). However, it is unclear if this dietary mitigation strategy would also provide beneficial effects on growth performance in pigs without a viral challenge. Therefore, a third experiment was conducted (Chapter 3) to determine if an increased SID Lys:ME ratio would improve growth performance in pigs undergoing an MHP challenge in late finishing. *Mycoplasma hyopneumoniae* is a bacterial

pathogen commonly seen in the U.S. swine industry, as it antagonizes growth rates resulting in increased market weight variation (Pieters and Maies, 2019). In an effort to control MHP occurrence, pigs are commonly vaccinated for MHP as a wide array of vaccines are commercially available. Vaccination has shown to reduce clinical signs and lung lesions thus improving growth performance; however, studies have also shown that vaccination may result in limited reductions of MHP transmission (Maes et al., 2018). In the experiment outlined in Chapter 3, the same pigs used in experiment 2 were utilized for a 40 d study prior to marketing. At ~80 kg BW, one barn was inoculated with aerosolized MHP infected lung homogenate, while the second barn remained MHP negative, serving as the control non-MHP inoculated group. Within barn, one of two dietary treatments were assigned resulting in 12 pens per dietary treatment per MHP status. The two treatments were: 1) A control diet formulated to contain 1.95 g SID Lys:ME (representing 100% Lys:ME) and 2) A high Lys:ME (120% ratio) diet containing 2.34 g SID Lys:ME achieved via the increased inclusion of SBM.

Overall, during the 40 days following the MHP challenge, the 120% Lys:ME ratio had no effect on growth performance or end BW in late finishing pigs in comparison to control fed pigs, in either MHP challenged or MHP naïve pigs. Protein deposition (**PD**) in swine is dependent on various factors such as genetics, BW, sex and environmental stressor present (NRC, 2012). Various swine genotypes have a limit to daily protein deposition (PD_{max}) and deposit the excess dietary protein in the body as lipid (Moughan et al., 2006). Consequently, leading us to hypothesize that the results of experiment 3 may be attributed to the late finishing pigs reaching their PD_{max} during the experiment. Thus, feeding an increased Lys diet would not be as beneficial as in early growing stages (experiments 1 and 2) when PD is more prevalent. Additionally, the pigs in Chapter 3 had received vaccination against MHP prior to weaning,

possibly reduced the impact of MHP inoculation on pig growth performance. However, vaccines available for MHP have had varying vaccine efficacies reported (Tao et al., 2019).

Commonly in today's swine production, herds are vaccinated against various diseases such as PRRSV and MHP to help mitigate the negative effects anticipated in the event of a disease challenge. Commercially available PRRSV vaccines are either modified live vaccines (MLV) or killed autogenous vaccines developed from field isolates, which have been demonstrated to vary widely in efficacy (Osorio et al., 1998 ; Mavromatis et al., 1999; Oh et al., 2019). In experiment 1 (Chapter 2), one barn received an MLV PRRS vaccine (Ingelvac PRRS® MLV, Boehringer Ingelheim, St. Joseph, MO), which resulted in seroconversion prior to challenge with virulent virus improved survivability during an experimental PRRSV challenge as PRRSV antibodies were detectable prior to inoculation. Vaccines applied to mucosal surfaces that target mucosal immune cells are also being develop; however, limited reports of their efficacy are available. In experiment 2 (Chapter 3), half of the pigs received a killed mucosal PRRSV vaccine (Aptimmune Barricade Mucosal killed autogenous PRRS vaccine, Dimond Animal Health, Des Moines, IA); however, no differences were observed between vaccinated and non-vaccinated pigs during the experimental PRRSV challenge.

Additionally, in Chapter 2 and prior to experiments 2 and 3, all pigs were fed two phases of low, medium and high SBM diets (phase 1: 15%, 25% and 35% and phase 2: 25%, 35% and 45% SBM). Soybean meal is a highly palatable protein source that is widely used when formulating diets in the U.S. as the AA profile of SBM fits well with that of corn and other cereal grains. However, in newly weaned pigs recommended limitations of SBM are often advised due to antigenic properties that can cause hypersensitivity to soybeans (Li et al., 1990; Li et al., 1991; Song et al., 2010). The hypersensitivity to soy protein is commonly greatest from 1

to 10 days post weaning, after which pigs develop an “immune tolerance” (Barratt et al., 1978). Reductions in growth rates, nutrient digestibility and intestinal villus height have been seen as a result of high levels of SBM (Dréau et al., 1994; Jones et al., 2010; Song et al., 2010). However, contradicting studies have shown no reduction in pig growth with increased SBM in early nursery diets up 22.5% inclusion (Friesen et al., 1993; Moran et al., 2017). In agreeance, in Chapter 3, during the nursery phase no reduction in ADG or end BW was observed as a result of medium or high SBM diets compared to that of control. In an effort to reduce soy protein present in the diet (i.e. reduce hypersensitivity response), highly digestible animal protein sources (ex. fish meal or animal plasma) are commonly included in nursery diets to stimulate ADFI and ADG (Jones et al., 2010; Sulabo et al., 2013). However, in phase 1 of the current study, the high SBM diet which contained the lowest inclusion of specialty proteins and highest level of SBM, improved ADFI compared to low and medium SBM diets. Although, not the objective of this thesis, in regards to growth these results indicate that SBM inclusion levels in nursery diets could possibly be higher than previously thought as overall ADG, ADFI and G:F did not differ across dietary treatments at the conclusion of the nursery period.

The data reported in this thesis offers support that increasing dietary Lys:ME to 110% to 120% of requirement improves growth performance and feed efficiency in experimentally challenged PRRSV pigs (Schweer et al., 2018a). As expected these data confirmed that in the event of a stressor such as a PRRSV challenge, AA requirements may change due to increased metabolic activity and repartitioning of nutrients away from lean tissue accretion, indicating the importance and impact that feed intake has during a disease challenge. Additionally, data from this thesis has confirmed that pigs continue to exhibit their ability to eat to their energy needs during a PRRSV challenge. However, the PRRSV challenge seen in experiment 2 (Chapter 3)

was milder than the PRRSV challenge in experiment 1 (Chapter 2), which resulted in moderate reductions in growth performance parameters, consequently direct comparison between the two studies is not warranted. When comparing intramuscular to intranasal route of PRRSV inoculation, Yoon et al. (1996) reported a more uniform immune response in intramuscular inoculated pigs in comparison to intranasally inoculated pigs. Thus, a future direction to research may be administering a PRRSV challenge in a similar manner to experiment 1 (i.e. intramuscularly); however, evaluating the effects of a 120% Lys:ME diet achieved via a fibrous feedstuff as seen in the HF diet in experiment 3. This research would allow us to further evaluate the effects of utilizing a fibrous feedstuff to achieve a 120% Lys:ME ratio in PRRSV challenged pigs.

Additionally, further research is also needed to explore possible dietary strategies to improve growth performance in pigs undergoing common bacterial infections, such as MHP, *Escherichia coli*, *Brachyspira hyodysenteriae*, *Lawsonia intracellularis*, or *Streptococcus suis*, in which pathogenesis of the infective agent varies greatly between bacterial infections. In experiment 3 (Chapter 3), a SID Lys:ME ratio representing 120% of requirement was fed to both MHP infected and MHP naïve pigs, with no difference in performance observed across dietary treatments. Consequently, leading us to hypothesize that nutrient requirements of pigs undergoing a specific bacterial challenge may differ from that of pigs undergoing a PRRSV challenge. Previous work has shown that growing pigs undergoing a co-challenge of MHP and *Lawsonia intacellularis* resulted in reduced feed intake (Helm et al., 2018). This response in part can be attributed to the increased release of inflammatory cytokines such as interleukin (**IL**) -1β, IL-6 and tumor necrosis factor (**TNF**) –α that occurs during a MHP infection (Escobar et al., 2002). In bacterial infections causing increased release of appetite suppressing cytokines, the

reduction in feed intake may be a protective mechanism as mice infected with *Listeria monocytogenes* (i.e. bacterial challenge) nutritionally supplemented had increased mortality, while mice infected with influenza virus had increased survivability as a result of nutritional supplementation (Wang et al., 2016); as this work also aligns with Murray and Murray (1979). Thus, feeding a diet containing Lys above requirement during a bacterial challenge may not hold the same beneficial effects as previously seen in virally challenged pigs.

In conclusion, this thesis validates that during a controlled PRRSV challenge (with a concurrent natural PCV2 infection), increasing SID Lys:ME to 120% in grower pigs aids in the mitigation of negative growth performance associated with mixed infections including PRRSV challenge (Schweer et al., 2018a). Additionally, the ability of pigs to alter their voluntary feed intake to meet their energy needs was expressed during a health-challenge. Thus, increased feed intake was observed in pigs fed a diet with reduced dietary energy, which translated to an increase in ADG and end BW. This work is important to determining the nutrient requirements of health-challenged pigs in the swine industry today, to better optimize nutritional recommendations for pigs encountering a viral challenge. However, no benefit was observed in pigs challenged with MHP in late-finishing fed an 120% SID Lys:ME diet, as the severity of the challenge was mild. Further research is needed to evaluate possible nutritional mitigation strategies to better feed pigs challenged with various endemic bacterial and viral pathogens. Altogether, these findings further emphasize the importance of understanding and defining nutrient requirements of health-challenged pigs.

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